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BACTERIOLOGY

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This number of the Bulletin is devoted to reviews of recent work relevant to the study of virus, rickettsial, and bacterial disease. It is not intended to be a comprehensive statement of recent bacteriological work, but is concerned with subjects which are thought to be of special interest at the present time. Work on chemotherapeutic and antiseptic drugs is not included, but will be the subject of a later number.

The first article is the fifth in a series of occasional articles on the development of medical studies in Britain. The author, SIR JOHN LEDINGHAM, *contributed this article a few months before his death on October 4th, 1944. Dr. G. F. Petrie, of the Lister Institute, read and corrected the proofs of the article and has contributed the following short note on the author:*

"The recent death of Sir John Ledingham has removed a distinguished figure from the ranks of British bacteriologists. His life-long association with the Lister Institute, London, of which he was the director from 1930 until his retirement in 1943, gave him abundant opportunities of keeping in touch with research work in Britain and in other countries. He maintained friendly contact with many of the leaders of medical research throughout the world. His outlook and temper were, indeed, international, and he did much to foster amicable and helpful relations with men of science everywhere, as is witnessed by the notable part he played in the three international congresses of microbiology of the past decade; he was president of the second congress when it was held in London in 1936, and an honorary president of the third congress which met in New York in 1939 at the outbreak of the war. His own contributions to science gave evidence of an unusual width of knowledge, and include studies in pure bacteriology, haematology, immunology, pathology and virus agents. The article which follows bears testimony to the keen interest that he always took in the achievements of the past."

Ledingham had been emeritus professor of bacteriology in London University and bacteriologist to the Lister Institute for 38 years. He was associate editor of the Medical Research Council's "System of Bacteriology" and was appointed a member of the Council in 1934. He was author with Sir Joseph Arkwright of a book on "The carrier problem in infectious diseases" (1912). Further particulars of Ledingham's scientific career are to be found in obituary notices published in the British Medical Journal (1944, **2**, 514) *and Lancet* (1944, **2**, 550).

DR. C. H. ANDREWES *is well known as one of the foremost authorities on virus disease. He obtained his medical education at St. Bartholomew's Hospital, London, where he subsequently had several resident clinical appointments. From 1923–25 he was assistant resident physician at the Hospital of the Rockefeller Institute, New York City, and since 1926 he has worked at the National Institute for Medical Research, Hampstead (London), where he has been engaged almost entirely in the study of viruses, especially their immunology, their relation to tumours, and the influenza viruses. In 1933 Andrewes discovered, with Professor Wilson Smith and the late Sir Patrick Laidlaw, that influenza was due to a virus which would infect ferrets and mice. This important discovery was the beginning of the scientific study of the difficult problem of influenza.*

The name of DR. A. FELIX *will be familiar to all readers from his association with the Weil-Felix reaction for the diagnosis of typhus fever* (1916). *In more recent years Felix has established the value of this and similar reactions in the differentiation of rickettsioses throughout the world. He has also been responsible for work on the antigenic analysis of bacteria, in particular the recognition of the H, O, and Vi antigens. This work has opened a chapter in serology and provided new methods of distinguishing closely-allied bacterial species and of dealing with problems of passive and active immunity. Since 1927 Felix has been a member of the scientific staff of the Lister Institute from which he has, during the war, been seconded to the Emergency Public Health Laboratory Service (Medical Research Council).*

DR. R. CRUICKSHANK *has been pathologist in charge of a Group Laboratory in the London County Council Hospital and Medical Services since 1936. He was lecturer in bacteriology in the University of Glasgow and bacteriologist to the Glasgow Royal Infirmary from 1928 to 1936. He received his early training in the bacteriology departments of Glasgow and Aberdeen Universities and did 3 years' clinical work in paediatrics and fevers. He has contributed to the literature on intestinal flora and intestinal infections, puerperal sepsis and the bacteriology of the genital tract, scarlet fever, pneumonia, pertussis and air-borne infection. He gave the Milroy Lectures of the Royal College of Physicians on "Pneumococcal infections" in 1933 and was a Chadwick Lecturer in 1943. He is joint-editor of "Control of the common fevers" (1942) and is a member of the Medical Research Council committees on hospital cross-infection, protein requirements, and measles prophylaxis.*

He has played an important part in the revival of interest in the mechanism and control of infections acquired in hospital, and is thus exceptionally well qualified as author of a historical review of this subject.

PROFESSOR A. A. MILES, *who contributes a review of modern views on the same subject, was educated at Cambridge and St. Bartholomew's Hospital. He was demonstrator in bacteriology with the late Professor W. W. C. Topley at the London School of Hygiene* (1929–31), *and then demonstrator in pathology at Cambridge until 1935. He then became Reader in bacteriology at the British Postgraduate Medical School, London, until he was appointed professor of bacteriology at University College Hospital Medical School (London University). For the past 5 years he has also been pathologist-in-charge of the London Sector IV of the Emergency Medical Service (Ministry of Health). His scientific activities have been mainly concerned with the immunological problems arising from antigenic analysis of bacteria. As a result of the war he has been involved in the study of cross-infection of wounds, but his interests are more in the general problems of epidemiology of wound-infection, both in war wounds and in industrial wounds, and in the special problems of infection*

with anaerobic bacteria. In connection with industrial wounds, he has recently worked at the Medical Research Council's Wound Infection Unit attached to the Birmingham Accident Hospital.

DR. W. J. T. MORGAN *is a member of the staff of the Lister Institute and Reader in biochemistry in the University of London. A note on his work appeared in Vol.* 2, *No.* 8–9, *of this Bulletin, to which he contributed a review on the "Occurrence and nature of human blood-group substances".*

DR. R. ST. JOHN-BROOKS *is curator of the National Collection of Type Cultures, which he describes and discusses in his article. He is permanent secretary of the Nomenclature Commission, and secretary of the National Committee for Great Britain and Northern Ireland, of the International Association of Microbiologists. He is also secretary of the recently-formed Medical Research Council Committee on Research in Medical Mycology and joint-secretary of the new Society for General Microbiology. Earlier he was special bacteriological investigator to the Local Government Board* (1911), *special sanitary investigator to the Governments of the Windward and Leeward Islands* (1912–14), *secretary to the Commission for Plague Investigation in India* (1914–15), *and specialist in bacteriology to the County of London War Hospital* (1915–19) *and to the Royal Army Medical College, Millbank* (1919–20). *He was secretary to the Second International Congress for Microbiology (London,* 1936) *and president (section* 1) *of the Third International Congress at New York* (1939).

REVIEW OF SELECTED PAPERS. *Contributions to this section have been received from Professor A. W. Downie, Professor L. P. Garrod, Dr. J. C. Cruickshank, Dr. M. A. Jennings, Dr. D. McClean and Dr. A. Macdonald*

SPECIAL CONTRIBUTIONS

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THE DEVELOPMENT OF MEDICAL STUDIES IN BRITAIN: V. BACTERIOLOGY

the late SIR JOHN C. G. LEDINGHAM, C.M.G., F.R.S.

The purpose of this article is to sketch the development of the science of bacteriology in Britain from its early days some seventy years ago, when a few scattered workers were striving to gain acceptance for the germ theory of infectious disease, to the world-wide and intensive researches of modern times with numerous workers contributing to knowledge which is constantly being applied to the solution of public health problems. Nowadays, increased and speedy intercommunication between men of science through special societies, scientific journals and international congresses has tended to make the research programmes of individuals and teams in different lands more or less similar, at least in general scope. The layman, too, by means of the radio, popular digests, and the public press, has now many opportunities of becoming acquainted with the powers of bacteria for good or ill and the part these play in a truly astonishing variety of natural processes.

An appreciation of bacteria as living entities fulfilling definite functions in nature came long after the demonstration in the seventeenth century of his "little animals" by Leeuwenhoek of Delft, who detected them by aid of his simple hand-made lenses and described his observations in a long series of letters to the Royal Society of London of which, in the year 1680, he was made a Fellow.

The real commencement of the bacteriological era dates from the fifties and sixties of last century, when Louis Pasteur, after finally disposing of the abiogenesis theory which had been a matter of controversy for a hundred years and more, turned his attention to the study of fermentation processes as observed in the manufacture of wine, beer and vinegar, and demonstrated conclusively that living and multiplying yeast cells were responsible for the observed phenomena. Others before him had come very near the truth, but the novelty of Pasteur's work and the failure of otherwise highly distinguished men of science of the time to comprehend it, was attested by the obstinate opposition of such men as Liebig and some of Pasteur's own compatriots. It will be remembered that Pasteur's final demolition of the abiogenesis belief failed to convince the British scientist, Charlton Bastian (1837–1915), who never ceased to maintain his own point of view.

Incredulity, indeed, was by no means confined to the ignorant and uneducated, as Lister also experienced when his antiseptic principle was first given to the world in 1865–67 at a time when Pasteur had turned to the investigation of silkworm disease. Before Pasteur's work on fermentation, studies of the larger fungi had been made and were greatly helped by improvements in the compound microscope in which British microscopists, notably Joseph Jackson Lister, father of Lord Lister, had taken a most important part. These studies had led to the recognition in the eighteen-thirties and -forties of fungal parasites in association with certain skin diseases of man such as ringworm and favus. In 1865 the association of the much smaller bacteria with infectious disease had not really been made an object of experimental enquiry, though this idea was certainly present in the mind of Pasteur as an extension of his experience with diseases of beer due to the work of wild yeasts. It was not, however, until the late seventies and early eighties that he was to make his great contributions to preventive medicine.

Achievement of Lister as a Bacteriologist

In 1865, when Lister, then Professor of Surgery in Glasgow, learned, largely by chance, of the work of Pasteur showing the dependence of the fermentation processes studied by him on the metabolism of the yeast cell, he was, so we are told, at once inspired with the notion that the "putrefactive" processes which he had sadly noted in surgical wounds and upon the causation of which he had long reflected, might also be due to the work of living microscopic agents. The revelation came to him in a flash and his reaction to it, like that of Pasteur when faced with a similar problem, was to attempt to control the morbid process without enquiring too closely into the nature and properties of the specific causative bacteria. Lister and Pasteur were essentially early exponents of preventive medicine, the one seeking to attack directly the presumed parasites that cause the festering of wounds, the other to immunize the host against the risk of an attack by invading germs in a virulent form. It is, indeed, remarkable that each of these principles has proved its worth in its own sphere of action, that of Pasteur having abundantly confirmed and extended the work of Jenner, on which Pasteur's use of artificially attenuated cultures was admittedly based.

Lister's antiseptic principle took root slowly, as is well known, and with its full recognition as an essential element of good surgery, its tardy universal adoption now gives us merely cause for wonder. Did Lister's discovery of the antiseptic principle make him a bacteriologist? In 1865 he knew little or nothing of bacteria and their ways beyond the fact that their access to wounds could be prevented by the use of such chemicals as carbolic acid, but Lister was not content with this knowledge. He resolved to make himself a bacteriologist quickly—and in the early years that followed his discovery of the antiseptic method, while a professor of surgery in Edinburgh, he spent the spare hours he was able to snatch from his professorial duties in an intensive study

of bacteria and fungi. He invented for his own use a complete bacteriological armamentarium together with ingenious accessories which enabled him to study both fungi and bacteria with a high degree of accuracy and under sterile conditions. Sir Charles Martin has, indeed, remarked in the course of a Lister Memorial Lecture " I doubt whether bacteriologists are aware that Lister invented all their methods before 1870 ". He made his mistakes both of interpretation and of technique but he was always ready to acknowledge them and to improve his methods. Moreover, by 1874, he had established a correspondence with Pasteur which was profitable to both. The fermentation and curdling of milk deeply interested Lister and he made a study of the factors which are involved in the process. With the aid of an ingenious syringe which he invented and which was capable of delivering minute quantities of fluid, he succeeded in securing a pure culture of the organism mainly responsible for the reaction, namely, *Bacterium lactis*. This is believed to be the first pure culture obtained of any organism. The great discovery of the use of solid media as an aid to the isolation of pure cultures of organisms was yet to come. It was made by Robert Koch (1843–1910), whose conclusive proof that *Bacillus anthracis* was the cause of anthrax appeared in 1876, and was soon followed by his experimental demonstration of micrococci as causative agents of wound sepsis. In the year 1881 Koch demonstrated his solid-culture method at the International Congress of Medicine in London in the presence of Pasteur and Lister and we can, even at this date, understand the surprise and pleasure of these two pioneers when they realized the importance of the discovery for the future of bacteriological practice. It has been remarked that in the rapid development of bacteriological knowledge which followed Koch's early discoveries, neither British nor American workers took any large share, and in a certain sense this is true and will be explained later. Nevertheless, in the opinion of the writer, had Britain possessed no exponent of bacteriology but Lister at the commencement of the bacteriological era, this country would have been adequately represented. The truth is that Lister's achievements in bacteriology have long been overshadowed by the pre-eminence of his work for surgery. Between the years 1873 and 1881 he communicated to the press only six substantial papers on his bacteriological pursuits, which were carried on at night and in the early morning when he was free from the preoccupations of his routine work

In a summary of British achievements in the sphere of bacteriology at this early date, one cannot omit mention of the work of John Tyndall (1820–1893) whose observations and experiments on the floating matter of the air in relation to putrefaction and infection appeared in 1881. Lister abandoned the carbolic spray as an aid to antisepsis in 1887, when he was satisfied that the organisms in dust played little or no part in the causation of wound sepsis, and that in any event the spray was powerless to destroy them. In his address to the Surgical Section on the International Medical Congress held in London in 1881, Lister made the remark, " It must be distinctly borne in mind that the spray is, beyond all question, the least important of our antiseptic means, and that the circumstance that a surgeon does not happen to have a spray-producer at hand is no excuse whatever for abandoning the attempt to obtain aseptic results." He was, in fact, convinced that the organisms most to be feared were those on the skin of the patient and the hands of operators and attendants. At the present time, with the knowledge we possess of the frequent presence of specific pathogenic organisms discharged into the air from septic dressings or expelled from the throats of doctors, nurses or patients, physicians and surgeons are finding it very important to take steps to control this additional source of infection in hospital wards. The carbolic spray which Lister had rightly abandoned as useless has its modern counterpart in the attempts to destroy infective germs and ultramicroscopic agents of disease in the atmosphere of rooms by aid of disinfectants in " aerosol " form.

Lister as a Pioneer in Immunology

Lister, from his earliest approach to the surgeon's calling, had pondered much over the sequence of changes, whether morbid or otherwise, that take place in wounds and more particularly over the probable factors that make possible the healing of wounds under conditions permitting the access of saprophytic germs, for example, from the air. He had come to the conclusion that blood must possess some peculiar property of destroying such germs. This idea he put to the test by exposing fresh blood to air in sterile vessels when he found that putrefaction did not readily take place. A more elaborate and convincing proof came from an experiment in which he withdrew blood from the jugular vein of an ox into tubes, allowed it to clot, and added to the exuded serum tap-water in varying amounts. It was found that even at the temperature of the body, putrefaction did not take place when as much as eight drops of tap-water were added. The addition of putrid blood in minute quantities also failed to infect, nor did the addition of dust cause any putrefactive change within a reasonable period of time. He had thus independently demonstrated the presence in serum of a bactericidal agent which a few years before had been postulated by Lewis and Cunningham and later had been more elaborately studied by Fodor (1886) and by Nuttall (1888). This property of serum is almost certainly of great importance in natural defence. Further, in the course of experiments performed by Lister at the École Vétérinaire, Toulouse, he had carefully noted the changes which took place in vascularizing clots artificially produced in the jugular vein of an ass, and had observed the appearance of multitudes of " new living elements " whose function was a mystery to him until Metchnikoff's work on phagocytes appeared towards the close of the eighties.

Rapid Advances after Koch's Early Discoveries

The decade 1880–90 witnessed a succession of spectacular discoveries of disease agents, largely owing to Koch's having placed in the hands of students of bacteriology an equipment and a scheme of technical procedure which greatly facilitated the isolation and recognition of the bacterial agent responsible for the disease under study. Many authorities would date the commencement of modern bacteriology from this period. Pupils flocked to Koch's Institute in Berlin from his own country, and from many foreign countries including Britain, to understudy the master and subsequently to disseminate the new knowledge. At this time the output of newly discovered agents of infectious diseases was essentially the work of Koch or his pupils. In Britain there was no such centre of research. This had to await the return of students who had worked under Koch or elsewhere in Germany and the wide diffusion by translations of outstanding bacteriological texts. Lister was too occupied to found a school of bacteriology, though he alone was capable of doing so. Watson Cheyne, the surgeon who accompanied Lister from Edinburgh to London in 1877 and who, like his chief, had an active interest in bacteriological problems that were the concern of the surgeon, studied and worked for a time with Koch and later did excellent service by making known to English readers, through his books, the achievements of the new science. Edgar Crookshank (1858–1928), who studied in various German laboratories and later established the first bacteriological laboratory in London at King's College, produced in 1886 a *Manual of bacteriology*, which still occupies an important place among the early bacteriological handbooks and is particularly valuable for its comprehensive references to papers up to that time in all branches of bacteriology. Lister and Watson Cheyne, with Alexander Ogston (1844–1929) of Aberdeen, made a trio of bacteriologist-surgeons whose original work worthily represents British effort in the field of bacteriology at this early period. Ogston during 1880–83 carried out fundamental research on the micrococci present in abscesses, thus completing and amplifying Koch's contribution to the subject in 1878. By cultural methods, a special staining technique, and inoculation of cultures into experimental animals, he proved in a masterly manner that abscesses in man of the acute type invariably contain micrococci and that these latter are of two kinds and are distinguishable by their manner of arrangement in stained films of the pus. He distinguished the *Streptococcus* of Billroth from another form, resembling bunches of grapes, which he named the *Staphylococcus*. Ogston's work greatly clarified the hitherto rather confusing picture presented by coccal parasites in these abscesses and in septic processes generally.

Lister's boldly conceived principle of antisepsis, which was based on the firm belief that " putrefaction " of wounds must surely, like other processes studied by Pasteur, be the work of micro-organisms, became in Britain, as was natural in the first instance, the province of surgeons eager to acquire a more intimate knowledge of the types of bacteria which are

liable to frustrate their surgical skill and endeavour. Outside the ranks of the surgeons there were at the time (the eighties) in Great Britain few if any trained pathologists with the exception of Greenfield of Edinburgh, who were attracted to the new study. The reason is that a chair of pathology, except in Scotland, did not then exist in Britain. The most prominent representatives of experimental medicine in Britain during the seventies were physiologists, headed by the veteran William Sharpey. Their work was in the main confined to histology and microscopy, but after the formation of the Physiological Society in 1876, followed two years later by the foundation of the *Journal of Physiology*, the experimental aspect of the subject became and has continued to be, in Britain, the research field of a long succession of distinguished exponents whose names are household words throughout the world. Cambridge University and University College, London, have long been, perhaps, the most favoured centres of their labours. The incursion of physiologists into bacteriology, at a time when reports of the early discoveries of specific micro-organisms in infectious disease were being made, was not a particularly happy venture, for the new subject of enquiry demanded a novel and difficult technique, and the correct interpretation of bacteriological findings derived from cultivation methods required considerable experience which few then possessed. It is noteworthy that the physiologists paid great attention to the probable mechanism, in the light of purely physiological principles, of the pathological lesions they found at necropsies (of suspected cholera cases), irrespective of whether the cultures yielded Koch's vibrio or not. The bacteriologist of to-day would welcome collaboration with the physiologist, for there are many aspects of bacterial activity, such as the nature of the interactions of host and parasite at their earliest contacts, which would be likely to receive illumination from his special methods of study. Their partnership could hardly, nowadays, be followed by such a strange happening as Lister's warning against the easy acceptance in 1881 of Ogston's work on the causative role of the staphylococcus in acute abscesses, on the ground that the phenomena of inflammation which he had carefully studied as a young postgraduate under the inspiration of Sharpey, might conceivably include suppuration as an end-result of nervous influence on the blood vessels. Lister attached cardinal importance to this influence and attributed to it such results of counter-irritation therapy as he had observed. Ogston replied effectively to the criticism and, needless to say, Lister quickly revised his opinion.

Modern Period

Towards the close of the 19th century it became obvious to men of vision in Britain that a specially trained cadre of bacteriologists was absolutely essential to ensure the future progress of the science in this country. The marvellous work of Pasteur during his declining years, and in particular his studies on the control of rabies (1884), the novel and fascinating researches of Metchnikoff, and the interest excited by the discovery of diphtheria and tetanus toxins and their corresponding antitoxins, all served to emphasize the necessity of concentrating bacteriological research in special institutes and in departments of pathology of the universities and of the large voluntary hospitals. In 1891 the *British Institute of Preventive Medicine*, later to be renamed the *Jenner Institute* and finally the *Lister Institute*, was incorporated and, commencing its research career in 1893, has now half a century of performance to its credit in many fields of experimental medicine including that of bacteriology and microbiology generally. During this time it has afforded research facilities to many workers both from the British Commonwealth and from foreign lands.

The circumstance that the staff of a research institution usually includes experts in the basic sciences such as biochemistry and biophysics, has the effect of raising the quality of the work, always provided that the fullest liaison exists between the workers in the various fields. The lack of clinical material may, at times, be seriously felt and should, if at all possible, be made good by the provision of research hospital-units attached to the institution. There can be little doubt that in the future the facilities offered by research institutions to workers from abroad will be greatly extended, and that this will operate to the certain benefit of science and to the cause of international amity.

Some thirty years ago, under the terms of the National Health Insurance Act (1911), financial provision was made for medical research, and was followed by the formation of the *Medical Research Committee* (later the *Medical Research Council*) working under the Privy Council and responsible not only for the conduct of medical research in a special institute (*National Institute for Medical Research*) but also for the financial support of research in many branches of medicine undertaken by selected workers throughout the country. The value of the services of the *Council* to medical research, including bacteriological science, has been incalculable, and the investigations of its experts in the *National Institute*, together with the reports of its special enquiries and surveys (*M.R.C. Special Report Series*), have long been known and appreciated throughout the world.

Research and Teaching

Bacteriological research has long been pursued in the pathological departments of all the British universities and many of the large voluntary and municipal hospitals. In addition, there have, for some time, been chairs of bacteriology in the great majority of the universities, where not only medical bacteriology, but also bacteriology as applied to agriculture and industry, is taught and studied. At Cambridge, since 1924 when pathology was included as a subject in Part II of the *Natural Sciences Tripos*, more than half the time of the course is devoted to bacteriology. The veterinary world possesses a number of institutions such as the *Royal Veterinary College*, the *Institute of Animal Pathology* at Cambridge, and the Veterinary Research Department of the Ministry of Agriculture, in which active work is conducted on bacterial and virus diseases of farm animals.

Recently-introduced equipment such as ultracentrifuges of the Svedberg and other types, electrophoresis apparatus, and electron microscopes, are now available in one or other of the various institutions I have mentioned above, so that the visitor from abroad who wishes to put his ideas to the test will have no difficulty in finding facilities adequate for his purpose.

Bacteriology as applied to hygiene, sanitation, epidemiological enquiry, and the diagnosis of infectious disease, has been practised in Britain from a very early period. During the present war facilities for applying bacteriological methods in the field of hygiene have been greatly augmented by the provision of many additional laboratories throughout the country under the Emergency Public Health Laboratory Service organized by the *Medical Research Council*.

At Cambridge University in 1875 the first Diploma in Public Health was established, and this example was followed by universities and licensing bodies. Bacteriology and serology have always formed an important part of the instruction for this diploma. Courses leading to a postgraduate diploma in bacteriology were established in the University of Manchester by the late Professor W. W. C. Topley in 1923 and served, no doubt, as the pattern from which Topley developed the courses in the *London School of Hygiene* for the academic Diploma in Bacteriology of London University.

Outside the purely medical fields there have, for many years, been departments in the large agricultural and dairy research institutes for the study of bacteriology and protozoology in relation to the soil, to plant diseases of bacterial and virus origin, and to the numerous problems of the dairy industry. I need mention only such institutes as the *Rothamsted Experimental Station*, known the world over for its services to agriculture, the *National Institute for Research in Dairying* at Reading, the *Hannah Dairy Research Institute* in Ayrshire, and the *Rowett Institute* in Aberdeenshire. Many industrial firms, or combinations of such, also have their research departments in which studies of bacteria, or it may be of fungi and protozoa, are conducted in so far as they concern the particular industry whether it be brewing, leather manufacture, or the search by chemical and pharmaceutical firms for new substances of chemotherapeutic importance. A long list of such firms could be cited.

Technical schools (polytechnics) in the larger cities arrange also for the teaching and study of this subject which, indeed, would seem to enter more and more into the register of commitments essential for industrial progress in many fields.

A subdivision of microbiology, and a very important one to which I have not yet alluded, is protozoology, particularly in its relation to animal diseases. It will suffice, however,

to state that the study of protozoa as causative agents of disease has long been intensively pursued in the great Schools of Tropical Medicine in London and Liverpool, the *Wellcome Bureau of Scientific Research*, and in the universities, for in this sphere of enquiry Britain can boast a line of pioneers, many of them of Scottish birth (e.g. Manson, Ross, Bruce and Leishman), whose names have been known throughout the scientific world for at least half a century. In recent years the term Microbiology has come to connote a study which treats of virus agents, protozoa and microfungi in addition to bacteria, and it is significant that three international congresses for microbiology, held respectively in Paris, London and New York during the past decade, adopted this convenient term and reported progress in all those subjects which are concerned with the activities of living microscopic and submicroscopic agents.

Literature

The purely specialist journals published in Britain which contain or may contain papers on microbiology (including bacteriology, virus research, protozoology, and mycology) are the following: the *Journal of Pathology and Bacteriology*, the *British Journal of Experimental Pathology*, the *Journal of Hygiene*, the *Journal of Dairy Research*, the *Annals of Applied Biology*, the *Transactions of the British Mycological Society*, the *Abstracts of the Society of Agricultural Bacteriologists*, and *Parasitology*, but many important papers have first seen the light in the *Proceedings of the Royal Society* (*Series B*), the *Lancet*, and the *British Medical Journal*; in recent years, *Nature*, like *Science* in the United States of America, has given early publicity in its columns to new or promising developments. The *Transactions of the Pathological Society of London*, whose long history came to an end when the Royal Society of Medicine was founded, the *Philosophical Transactions of the Royal Society of London*, and the *Quarterly Journal of Microscopical Science* contain many of the earlier contributions to bacteriology published in Britain during the last quarter of the past century.

The *Tropical Diseases Bulletin* and the *Bulletin of Hygiene*, now in their 41st and 19th volumes respectively, contain *inter alia* valuable abstracts and reviews of current microbiological research. Both are issued under the direction of the Honorary Managing Committee (appointed by the Secretary of State for the Colonies) of the *Bureau of Hygiene and Tropical Diseases*, Keppel Street, London, W.C. 1. [This Bureau also publishes, on behalf of the *Medical Research Council*, a *Bulletin of War Medicine*, now in its fifth volume.]

I cannot fail to allude here to at least five outstanding additions to general knowledge and to the history of bacteriological science, viz.: (i) *The history of bacteriology* by the late Professor William Bulloch, to which I have often had to refer while compiling this sketch; (ii) *A system of bacteriology in relation to medicine* in nine volumes, written by British workers and published between the years 1929–31 by His Majesty's Stationery Office under the aegis of the *Medical Research Council*; (iii) the very notable and comprehensive treatise, *Principles of bacteriology and immunity*, by the late Professor Topley and his colleague Professor G. S. Wilson of the *London School of Hygiene*; (iv) *Diphtheria: its bacteriology, pathology and immunology*, written by seven members of the Bacteriological Committee of the *Medical Research Council* and published in 1923 by His Majesty's Stationery Office; and (v) *Antony van Leeuwenhoek and his "little animals"*, by Clifford Dobell (London, 1932): a masterly treatise on the life and work of the discoverer of bacteria.

Some Twentieth-Century Contributions

It would be a difficult task to attempt in a short article any detailed survey of British bacteriological studies during the past half-century, but I may usefully draw attention to some notable advances dating from the commencement of the present century:

i. The contributions to immunology in the early years of the century, with particular reference to the prophylaxis of enteric disease. Such studies have since been actively pursued as the need for control of the manufacture and standardization of biological products, particularly therapeutic sera, became more and more acute, and as the urge arose to explore the mechanism of antibody-production, the chemical nature of antigen and antibody, and their manner of union (immunochemistry).

ii. The work of the Tuberculosis Commission, which revealed the importance of bovine tubercle bacilli as causative agents of tuberculosis in man.

iii. The experimental demonstration of the transmission of bubonic plague by the rat-flea.

iv. The work of the Mediterranean Fever Commission, which incriminated the goat as the reservoir of the causative agent, discovered in 1887 by David Bruce, and also the work of the *Royal Society*'s Sleeping Sickness Commission.

v. The pioneer researches into anaerobes and their differentiation carried out by a team of workers during and after the war of 1914–18.

vi. The new knowledge relating to bacterial variation during the first decade, and the later contributions on the antigenic constitution of the enteric and food-poisoning groups of bacteria.

vii. The work on experimental epidemiology by the late Professor W. W. C. Topley and Professor M. Greenwood at the London School of Hygiene.

viii. The elaboration of a trustworthy and efficient prophylactic agent against diphtheria (A.P.T.) now widely used throughout the world and emanating from the *Wellcome* Serum Laboratories.

ix. The differentiation and significance of the *gravis*, *mitis* and *intermedius* types of *C. diphtheriae* by workers of the Leeds school.

x. The contributions of the late Dr. F. Griffith and the late Dr. W. M. Scott, both victims of enemy action during the present war, to the serological typing of streptococci, and its application to such problems as the mechanism of origin of puerperal sepsis and ward infection.

xi. The contributions of biochemists to the study of bacterial metabolism and nutrition, commencing with the pioneer studies of the late Arthur Harden of the *Lister* Institute and continued there and also at the Biochemical Department of the University of Cambridge, the Department of Bacterial Chemistry of the *Medical Research Council*, the Bacteriological School of Leeds University and other centres.

xii. The discovery of bacteriophage at the *Brown* Institution by F. W. Twort.

xiii. Chemotherapeutic investigations in protozoal and bacterial disease, including the discovery of penicillin by Fleming at *St. Mary's* Hospital and the development of this discovery by Florey and his colleagues at Oxford; and the contributions to the chemotherapy of certain protozoal diseases by the late Professor Warrington Yorke and others.

xiv. The substantial contributions of British workers during the past two decades to our knowledge of virus diseases and of virus agents, particularly the discovery of a virus associated with influenza made at the *National Institute for Medical Research*; and the enquiries into the nature of virus bodies, such as those of vaccinia and smallpox, described originally by Buist in the eighties of the last century, independently rediscovered by the late Professor Paschen, but only in recent years in Britain rendered capable of isolation in a pure state and proved to be the actual causative agents of the diseases in question. These bodies, whether of plant or of animal origin, have now become the subject of intensive chemical and biophysical study both in this country and in the U.S.A.

This brief recital of notable advances must for the moment suffice. Further rapid progress may be expected in the future as the solution of the numerous problems presented by microbiology engages more and more the interest and assistance of experts in the basic sciences. A firmer liaison than now exists in Britain between workers in the various fields of microbiology would seem to be highly desirable for the discussion of common problems, and it is hoped that this desideratum may be realized in some effective way in the post-war period.

In conclusion, I cannot omit mention of the services rendered to bacteriologists in all spheres of work and in many lands by the *National Collection of Type Cultures*, located at the *Lister* Institute, with the financial support of the *Medical Research Council*.

Bibliographical Note

Reference may also be made to the following sources: *The collected papers of Joseph, Baron Lister*, 2 vols. (Oxford, 1909) [Lister's papers on pathology and bacteriology appear in Vol. 1, pp. 201–399].

Crookshank's *Manual of bacteriology* (London, 2nd ed., 1887) [dedicated to Lister].

Lord Lister, by Sir Rickman John Godlee (Oxford, 3rd ed., 1924).

The *Medical Research Council Special Report Series* [248 of these reports have now been published by H.M. Stationery Office; they include a very large number dealing with bacteriology and public health problems and first appeared in 1915. The *Catalogue of the National Collection of Type Cultures* is No. 214 of the series].

Sir Charles Martin's Harveian Oration, *Lister's early bacteriological researches and the origin of his antiseptic system*, appeared in *Med. J. Aust.*, 1932, **2**, 437.

Obituary notices of the earlier British workers in bacteriology are to be found in the *Proceedings of the Royal Society, Series B*, the *Journal of Pathology and Bacteriology* and elsewhere; for references, see Bulloch's *History of bacteriology*.

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VIRUS DISEASES OF MAN: A REVIEW OF RECENT PROGRESS

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The subject of virus diseases is now so extensive that a review such as this cannot attempt to cover it all. The writer must, therefore, confine himself primarily to the diseases affecting man and consider only those aspects in which he judges that the greatest progress has recently been made. No attempt will be made to go deeply into the large and controversial subject of the nature of viruses, and many other subjects such as the relationship of viruses to tumours will also have to be omitted.

New Biological Techniques

Viruses are known by their small size, their inability to grow on lifeless culture media, and their dependence for multiplication on a habitat within living cells, whether animal or vegetable. New methods for studying them, many of them expensive and complicated, have been evolved in recent years; but ultimately almost all work on viruses is limited by the need for a susceptible laboratory animal. Diseases of veterinary importance affecting cattle, sheep, horses or poultry can, if necessary, be studied by experiments on the affected species, though this is often a costly business; but for the study of human virus diseases a convenient small laboratory animal is of prime importance. Fortunately, the rabbit, the guinea-pig or the mouse can be infected with many important viruses, such as those of vaccinia, herpes simplex or yellow fever; but some human viruses will infect only rhesus or other species of monkey; examples are mumps, measles and trachoma. The only animal reported as being susceptible to the virus of the common cold is the chimpanzee.

Progress has been made in recent years by successful search for "new" laboratory animals, more convenient and less expensive than chimpanzees and monkeys. The use of the ferret by Laidlaw & Dunkin (1926) in their work on dog distemper introduced that animal to the virus-laboratory, and it has been widely used since the discovery (Smith, Andrewes & Laidlaw, 1933) that it can be infected with influenza. In 1939 Armstrong transmitted a strain of poliomyelitis virus to the cotton-rat, *Sigmodon hispidus*, a rodent commonly found in the United States and not unlike an ordinary brown rat but with longer fur. Since then cotton-rats have been profitably used for the study of many virus diseases. They readily breed in captivity—needless to say a most important asset for any candidate for the post of a laboratory animal. Other rodents such as golden hamsters (*Cricetus auratus*) and voles (*Microtus arvensis*) also breed readily and are likely to be useful; and trials with yet others are reported with increasing frequency.

Kunkel (1943) has lately emphasized the advances which have accrued from the introduction of new species of plant hosts into the studies of plant viruses, and it seems likely that the same will apply in the case of animal viruses, though greater difficulties are involved. There are human diseases almost certainly due to viruses but not yet transmitted to species other than man—such are herpes zoster, molluscum contagiosum, dengue, sandfly fever and infectious hepatitis. Workers have shown much zeal in attempting transmission to whatever in the way of a menagerie they could obtain. But many animal-viruses will only infect one host-species. It is likely that, in the case of some human virus diseases, we shall have to be content for the present with the information obtainable from experiments on human volunteers, and even that much knowledge we may have to forgo if the disease is too dangerous for experiments on man.

The new "laboratory animal" which has yielded by far the best dividends is the fertile hen's egg. Embryonic tissues, whether in the living egg or in tissue-culture, will support the growth of many viruses which will not survive in newly hatched chicks. Woodruff & Goodpasture (1931) introduced the technique of growing viruses on the chorio-allantoic membrane of hens' eggs, and Burnet (1933) further developed it, particularly by describing a method for making an artificial air-sac over the area of membrane which it was desired to infect. This new technique is now in use in almost every virus-laboratory. Some viruses will produce isolated countable lesions on the membrane, so that a virus-suspension can be titrated, much as are bacteria when inoculated on agar in a Petri dish; further, the technique can be applied to the titration of immune-sera and to other studies. Another advance came from Goodpasture's laboratories (Buddingh & Polk, 1939), and again this was developed by Burnet (1940): viruses were inoculated into the amniotic cavity of the developing embryo and thus almost directly into its respiratory tract. Cox (1938) first introduced viruses into the embryo's yolk-sac, and this technique is the basis of modern methods of cultivating the rickettsiae of typhus fever for vaccine manufacture. So, for various purposes, viruses may nowadays be injected either on to the chorio-allantoic membrane, or into the amniotic, allantoic or yolk-sac cavities or directly into the body of the small embryo.

Nor does this exhaust the use of the chick-embryo. Goodpasture & Anderson (1944) have sought to overcome difficulties due to a lack of species susceptible to human viruses, by grafting human skin or other tissues on to chick chorio-allantoic membranes and infecting them while they are surviving there. In this way, they have infected human tissues *in ovo* with the virus of herpes zoster, showing that they have done so by demonstrating characteristic "inclusion bodies" in the nuclei of infected cells.

Finally, minced chick-embryo is the favourite medium for growing viruses in tissue-cultures. Probably most viruses which are accessible to laboratory study at all, have been grown in tissue-cultures. For many, the simplest techniques suffice; it is only necessary to mince up some of the chosen tissue, suspend it in a modified saline (Tyrone's solution) in a suitable small glass vessel, add some virus and incubate at 37° C. In these instances the animal tissues are infected while merely surviving, not multiplying. But for other viruses, one has to add serum or plasma to the medium, to observe greater care as to its composition and to use the more elaborate techniques which ensure that the cells not only survive, but remain reasonably healthy and multiply. Propagation of viruses in tissue-cultures is proved by grinding them up and testing them for the presence of virus by animal-inoculation (while making due allowance for the possibility of mere survival), by finding characteristic pathological changes in cells such as "inclusion bodies" or by detection of alterations in the growth of the cultures.

Erythrocyte Agglutination by Viruses

Recently Hirst (1941, 1942)—and, independently, McClelland & Hare (1941)—noted when opening chick-embryos infected with influenza virus that erythrocytes from accidentally cut vessels of the chick-embryo were agglutinated when the blood flowed into the allantoic fluid. No such agglutination occurred in the case of uninfected embryos. Further work showed that it was, in fact, the adsorption of influenza virus itself on to the erythrocytes that caused the agglutination. Here was the basis of a very elegant and convenient technique for quantitative studies of influenza, and workers have not been slow to make use of it. Cells of adult fowls, and, rather less

conveniently, of other species such as guinea-pigs or human beings (group O) can be used. Virus can be estimated quantitatively and so can antisera, for in the presence of the appropriate antiserum specific agglutination of erythrocytes does not occur. Influenza virus, treated with formalin or stored for long periods until no longer infective, may yet retain agglutinating power for erythrocytes, but extracts of virus cause no agglutination. Study of the mechanism of this phenomenon may shed light on the way in which viruses infect cells. Apparently virus is adsorbed to erythrocytes, but after a period of time comes off again, is eluted, having during its attachment so altered the erythrocyte surface that the addition of fresh virus will no longer agglutinate the cells. Experiments with living ferret lungs (Hirst, 1943) indicated that virus was adsorbed to living susceptible cells just as to erythrocytes, but that spontaneous elution of virus did not occur: presumably the first attachment was succeeded by some other and firmer cell-virus relationship. On the other hand, in ferret lungs removed from the animal, virus was taken up, but later became detached, just as in tests with erythrocytes; the secondary stage, perhaps of penetration into the cell, seemed not to take place. Formalin-treated virus under these conditions was adsorbed, but could not be eluted.

Some other viruses, such as vaccinia, will agglutinate erythrocytes, but whereas influenza virus does so with cells of various animal species, vaccinia agglutinates only fowl-cells, but not the cells of every fowl (Clark & Nagler, 1943).

Interference Phenomenon

In some circumstances an animal cell may be simultaneously infected by two or more viruses, but such double infection is not always possible. There is evidence that sometimes, when a cell has sufficient contact with one sort of virus, it is for a time incapable of being infected with certain other viruses, which may be immunologically unrelated to the first. One virus is said to "interfere" with the action of another. The phenomenon is well known to students of plant viruses; as regards animal viruses it was first heard of (Hoskins, 1935) in connection with yellow fever; it was found that monkeys injected with the fatal viscerotropic strain of virus could have their lives saved by subcutaneous injection, at the same time or within a short period afterwards, of the neurotropic variant of yellow-fever virus. This variant does not kill monkeys when injected subcutaneously. Interference has been shown to occur between the viruses of yellow fever and Rift-Valley fever (Findlay & MacCallum, 1937a), between different strains of poliomyelitis (Jungeblut & Sanders, 1942), between different strains of influenza (Andrewes, 1942a; Henle & Henle, 1944), between the viruses of poliomyelitis and lymphocytic choriomeningitis (Dalldorf, Douglass & Robinson, 1938), between the viruses of western equine encephalomyelitis and St. Louis encephalitis (Duffy, 1944) and between two viruses affecting rabbits (Andrewes, 1940). The mechanism has been explained, at least in part, by experiments with two bacteriophages attacking *B. coli*. (There is every reason to consider phages as viruses which parasitize bacteria). Delbrück & Luria (1942) found that if even one phage particle had sufficient access to a bacterial cell, other phage particles of the same or another kind were frustrated in their efforts to infect the cell also. It was even found that phage inactivated by ultra-violet radiation could, after adsorption by the bacterium, still block the activity of other phage added later. The suggestion was made that the phage particle first added could preoccupy a key-enzyme in the bacterium, thus preventing the propagation of late-comers.

These findings have been followed up by Henle & Henle (1943, 1944) and by Ziegler & Horsfall (1944), who studied the power of one influenza virus to interfere with the multiplication of another within the allantoic cavity of chick-embryos; they also found that virus inactivated by ultra-violet radiation or otherwise could stop the activity of small quantities of living virus. All this work opens up new fields for thought and experiment. A new approach to the study of the mode of attack of viruses is clearly to be seen. The use of inactivated virus or attenuated live virus is now conceivable not as an immunizing vaccine in the ordinary sense, but as an immediately effective prophylactic agent of a new type. Perhaps most entertaining of all are speculations as to whether one virus may fail to spread epidemically in a community because of the presence of another virus inimical to it.

Chemical and Physical Studies of Viruses

Our discovery of how viruses act will naturally be closely linked with the discovery of what viruses are. There are now available new tools with the aid of which we are learning more and more about their nature. Physical methods are not only revealing the size, shape and other properties of viruses, but are being used to purify them so that we may obtain some information as to their chemical composition.

The sizes of viruses have been estimated by filtration, centrifugation and optical methods, in most instances with fair agreement. The simplest, most generally used method is filtration through gradocol membranes. These are tough, opaque collodion membranes whose preparation was described by Elford (1931). The use of different proportions of solvents made it possible to prepare at will membranes with pores of any desired diameter, from 3μ or so downwards, and of satisfactory uniformity. From the results of filtration, it is possible to deduce the size of the virus-particles, but it is necessary for workers to be aware of the factors introduced by the nature of the suspending fluid, the titre of the unfiltered virus, and so on.

Many techniques have been described for determining the properties of viruses by ultracentrifugation. Most dramatic have been the results obtained with the Svedberg analytical ultracentrifuge and modifications of it. The rate of sedimentation of purified virus-suspensions is determined by means of photographs taken while the machine is actually running, but knowledge of shape and specific gravity is necessary before size can be deduced. Optical methods give the most direct information about size and shape. Ultra-violet photographs have revealed much, and the electron-microscope is showing us yet more, though the latter suffers from the disadvantage that material for study must be dried and examined *in vacuo*.

By the combined use of these new instruments we are now in a position to give approximate figures for the diameters of nearly all viruses, from the 10–15 mμ of poliomyelitis and foot-and-mouth disease viruses up to the 300 mμ (0·3μ) of psittacosis. A virus related to poliomyelitis virus is now stated (Gard & Pedersen, 1941) to have an elongated needle-like shape—much as have tobacco-mosaic and other plant viruses; influenza virus is revealed by the electron-microscope to be oval or perhaps bean-shaped (Taylor, Sharp, Bland, Beard, Dingle & Feller, 1943); vaccinia and viruses related to it appear rectangular with areas of condensation of material within them, while others seem to be nearly spherical (Ruska, 1943; Rivers, 1943). Some bacteriophages have the appearance of rounded heads with slender tails attached—not unlike spermatozoa (Luria, Delbrück & Anderson, 1943).

Stanley's (1935) discovery that tobacco-mosaic could be crystallized gave the impetus to much work and to even more speculation as to what viruses were. So far no animal virus has been crystallized, though a number have been obtained in a state of considerable purity. The rabbit-papilloma virus (Bryan & Beard, 1940) seems to resemble many plant viruses in consisting, apparently, of pure nucleoprotein. Its simplicity of structure is also indicated by the fact that it behaves serologically as though consisting of a single antigen (Kidd, 1938). But most other animal viruses seem to be more complex. Vaccinia, for example (see Rivers, 1943), apparently has a surface-constituent which can become free and act as a soluble antigen, and so have several other viruses. That of vaccinia is itself complex, having a heat-stable and heat-labile constituent; moreover, vaccinia has probably got three or four other antigens in its constitution. Chemically, the virus contains nucleoprotein in which is incorporated a thymonucleic acid, and some lipid is apparently firmly bound in its structure. Copper and flavin are probably present also. Much work has been done in the effort to reveal enzymic activity on the part of purified vaccinia and other virus-particles, but the evidence is inconclusive, partly because enzymes from the host-cell seem to be rather easily adsorbed on to the surface of a virus. Few will now deny that vaccinia and other larger, relatively complex, viruses are analogous to bacteria, but have become simplified and have lost enzyme systems and other properties as a result of retrograde evolution and adaptation to a parasitic life. Not all are agreed, however, as to whether this explanation can still hold as one goes down the scale till one reaches the smallest, simplest viruses, including the plant viruses which can be crystallized.

Classification of Viruses

The time is not yet ripe to attempt a rigid classification of viruses, or to allot them generic and specific names. But it is worth considering briefly where to draw the dividing line between them and cultivable bacteria. No sharp line can be drawn by the application of any one criterion, whether this be size, shape, dependence for growth on living cells, staining properties or mode of transmission. But between the main body of viruses, which are clearly separate from bacteria, and the bacteria themselves, there stand two relatively well-defined groups:

(i) The *rickettsiae* which cause typhus and related diseases are smaller than most bacteria, nearly all completely dependent upon the interior of living cells for their livelihood, and their presence within cells at times leads to the formation therein of inclusion bodies. They differ from the majority of viruses in being relatively easily stained and in their varying morphology—bacillary forms are the general rule. All are insect-transmitted and most have an antigenic relationship to members of the proteus group of bacteria. Strong suspensions of some rickettsiae injected intraperitoneally into mice kill within a few hours by a kind of toxic action; but the "toxin" has not yet been separated from the living rickettsial bodies (Otto & Bickhardt, 1941). According to Zinsser & Schoenbach (1937), their metabolism in tissue-culture differs from that of viruses, but we have not yet sufficient information to say whether this is true. They are susceptible in some cases to chemotherapy but not by sulphonamides.

(ii) The "*LGV-psittacosis*" *group* has lately become defined mainly as a result of the work of Rake, Eaton and their collaborators (Rake, Eaton & Shaffer, 1941; Rake, Shaffer & Thygeson, 1942). It contains the viruses of lymphogranuloma venereum (LGV), psittacosis and related bird-borne infections (ornithosis; Meyer, 1942), trachoma, inclusion-conjunctivitis and pneumonia viruses affecting cats, mice and hamsters respectively. Viruses of this group have been recovered from a few cases of atypical pneumonia in man even in the absence of a history of contact with birds; the cause of the large majority of cases of "primary atypical pneumonia" is, however, obscure, though many claims to have recovered a virus have been published. Viruses of the "LGV" group are amongst the largest known; they stain, as do rickettsiae, with carbol-fuchsin by Machiavello's method and with Castañeda and Giemsa stains. They have a definite intracellular life-cycle, including large and small forms and morulae, as was first shown for psittacosis virus by Bedson & Bland (1934), but, unlike rickettsiae, they are at all stages rounded in shape. Like rickettsiae, the living virus-bodies injected in sufficient concentration kill mice by a quick toxin-like effect. All members of this group seem to be inter-related antigenically, as can be shown by cross-reactions in the complement-fixation test (Rake, Eaton & Shaffer, 1941). They can, however, be differentiated serologically when tests are made of the power of sera to neutralize the "toxin" (Rake & Jones, 1944). They differ from rickettsiae and from smaller viruses in that infections by many of the group can be favourably influenced by sulphonamides. They are not known to be insect-transmitted; but the agent of heart-water, which affects cattle in Africa, probably belongs to the group and is tick-transmitted; it has until lately been considered as a rickettsia.

Above these two groups stand the bacteria, whose properties need not be described except to point out that some of them are insect-transmitted, and some have not been grown *in vitro* on cell-free media. Below them are the other viruses, smaller in size, all obligate intracellular parasites, some of them insect-transmitted, of varying morphology, not so easily stained, not yet known to be susceptible to chemotherapy, many of them forming inclusion bodies within nucleus or cytoplasm, the smallest of them of relatively simple chemical composition. Viruses thus form a heterogeneous group and boundaries are nowhere easy to draw. But the writer finds it hard to agree with Rake that the "LGV-psittacosis" group should be excluded from the true viruses; it would seem more reasonable to include them and even the rickettsiae within the fold, though the latter are commonly considered as a group apart.

Chemotherapy of Virus Infections

It has been argued that the virus is a degraded bacterium, at its lowest reduced to naked nucleoprotein borrowing enzymes for its metabolism from the cell it parasitizes (Laidlaw, 1938). If so, chemotherapy has a hard task to find a drug which will penetrate the living cell and there stop the activity of the intruder without at the same time damaging the life-processes of the host-cell. Nevertheless something can be achieved in this direction. The intracellular rickettsiae of typhus can be attacked with success in laboratory experiments, though no drug of clinical value has yet emerged. Activity has been described in such varied substances as sulphones (Andrewes, King, van den Ende & Walker, 1944), toluidin blue (Peterson, 1944) and penicillin (Moragues, Pinkerton & Greiff, 1944). The toluidin blue apparently has a direct action on the rickettsiae, while the sulphones have not. In the LGV-psittacosis group, lymphogranuloma venereum itself is favourably influenced by various sulphonamides (MacCallum & Findlay, 1938); so, too, are trachoma, inclusion-conjunctivitis, pneumonitis of mice and heart-water of cattle. Psittacosis, ornithosis and pneumonitis of cats are apparently unaffected (Rake & Hamre, 1944). At present only a few strains of each virus have been tested, and it remains to be seen whether within one sort of virus there may be differences in susceptibility, such as may be seen within the group of streptococci. It is not certain, for instance, that psittacosis is always resistant. A number of claims to have found chemotherapeutic remedies for other virus diseases have not been confirmed when adequate tests have been made, and we must admit that, at present, we are in sore need of something to indicate promising new lines of attack. The need is the greater as serotherapy has almost nothing to its credit in the field of virus diseases. Sero-prophylaxis, as used to abort or attenuate measles, is, of course, of established value.

Transmission and Epidemiology

Influenza: Aerial transmission of virus infections is now thought to be brought about not only through the agency of droplets as ordinarily conceived, but even more by droplet-nuclei in the sense of Wells & Wells (1936). The droplets ejected during sneezing and loud talking are thought to evaporate, before they can reach the ground, to particles of the order of size of 10μ or less, too small to be rapidly acted upon by gravity and, therefore, capable of remaining suspended in the air for many hours. Such minute particles in the form of artificial virus mists have been shown to be capable of infecting ferrets and mice with influenza; further, Andrewes & Glover (1941) adduced evidence that droplet-nuclei were probably concerned in the natural transmission of influenza infection from one ferret to another when the animals were placed in cages several feet apart. Much work is being done on methods of killing organisms in the air by means of ultra-violet radiation or chemical aerosols. These methods are most effective against droplet-nuclei, much less so against dust-borne particles; so it is important to know just how viruses are carried. Promising results have been reported in the control of measles and chicken-pox by the use of ultra-violet radiation in schools and institutions (Wells, Wells & Wilder, 1942; Barenberg, Greene, Greenspan & Greenberg, 1942), and of respiratory infections in a children's convalescent home by propylene glycol mists (Harris & Stokes, 1942). If such results are confirmed, their success will itself be evidence as to the route of transmission of these diseases. Haemolytic streptococcal infections seem to be carried especially by dust, and methods of air disinfection, apart from dust-control, are not so likely to be effective in such circumstances. Influenza virus can survive on dust (Edward, 1941) but we do not yet know whether this is of practical importance.

Two influenza viruses are known, A and B, and these are antigenically unrelated to each other. Within each virus-species are strains differing antigenically from each other in minor degrees. How far such minor differences affect the epidemiological picture we do not know. Outbreaks of A- and B-influenza occur at times together, but more often separately. B is not known to have caused a major outbreak in Britain; epidemics, since 1933, when the virus was discovered (Smith, Andrewes & Laidlaw, 1933), have all been due to A. In this country they have tended to occur every other year with the bigger epidemics only every fourth year; the sequence has become a little "out of step" since 1940. In North America and Argentina also a biennial outbreak has been the general rule. Laboratory workers have usually failed to obtain any evidence at all of the activity of A-virus

in the 21 months or so between each period of activity, and it is of much importance for us to learn where it lies hidden in these periods. Shope (1941) has shown that, in the case of the closely related swine influenza, the virus may persist from year to year in the swine lung-worm. This has as its intermediate host the earthworm, and pigs at the beginning of a swine-influenza season may acquire infection by eating earthworms which carry lung-worms, these in turn bearing the virus infection. The story is rather more complex than there is space to describe now. A closely similar explanation is not readily conceivable in the case of the human disease, but we should be prepared for something bizarre to turn up. It has been suggested (Andrewes, 1942b) that between epidemics influenza virus may exist in human carriers, or as a cause of sporadic respiratory disease, but in a simpler "basic" form not recognizable by our present methods. Only at epidemic times would such a virus acquire, along with ability to spread in man, a pathogenicity for animals and ability to form in any quantity certain characteristic antigens. We have yet to explain, either along such lines, or by postulating yet undiscovered viruses C, D, E . . ., the frequent occurrence during A or B outbreaks, or separately, of many clinically similar cases, from which no virus can be recovered.

Poliomyelitis: The virus of infantile paralysis has been shown lately to be recoverable more easily from the faeces of patients than from nasopharyngeal washings (see Paul, 1941); it has also been found in sewage and on flies caught in the wild state (Trask, Paul & Melnick, 1943). The first portal of entry is doubtless the mouth and nose, but whether, therefrom, the virus commonly enters through the intestinal tract is still in dispute. It has been shown that poliomyelitis occurring in recently tonsillectomized children is more apt than ordinarily to be of the bulbar type (Aycock, 1942). Much interest has been aroused by the discovery of a biologically related virus (Theiler & Gard, 1940) which is present in the intestines and faeces of many normal laboratory mice. This only very exceptionally gives rise to spontaneous paralysis in mice which carry it, though it will readily do so on intracerebral inoculation. All these findings have caused us to re-orient our views as to the epidemiology and hygienic control of poliomyelitis.

Encephalitis: In the U.S.A., South America, Russia, and Japan, a number of forms of encephalitis have been recognized during the last decade, all transmitted by mosquitoes or ticks, and perhaps other arthropods. They are St. Louis encephalitis, Eastern, Western and Venezuelan equine encephalomyelitis, Japanese B encephalitis, Russian spring-summer encephalitis. Those named equine encephalomyelitis were so-called because of the losses they caused in horses, but they were later found to be causing disease in man, and also in birds. All the viruses concerned are of small size and readily transmitted to mice; there are antigenic relations between some of them, for example the St. Louis and Japanese B viruses (Casals, 1944). Insect-borne encephalitis in man has not been recognized in Britain, but should be watched for. Louping ill, which affects sheep in the hills along the Scottish border, is closely related to the Russian spring-summer encephalitis virus (Casals & Webster, 1944), and is known to be potentially pathogenic for man.

Vaccines

Few or no new principles have been established recently in the field of virus-vaccine prophylaxis. Living attenuated viruses are acknowledged to give the most durable immunity, but their uses for the control of human disease are few at the present time.

Vaccinia: The post-vaccinal encephalitis, which has been so troublesome, especially in the case of vaccination against smallpox, has been occurring much less frequently than a few years ago. We do not know why; its cause is still unsettled.

Yellow fever: The jaundice which followed vaccination against yellow fever with living attenuated virus in thousands of soldiers, especially in North America, has been a much more serious matter. Fortunately we do now know how to avoid that particular complication. An icterogenic agent was introduced in the human serum used for making or preserving the yellow-fever vaccine; the jaundice can be avoided by avoiding, as is readily possible, the use of serum in preparing the vaccine. This jaundice is but one example of "homologous serum jaundice," a disease most probably due to a virus present in some apparently normal human sera. It has occurred following the injection of mumps or measles convalescent serum, or after transfusions. Its identity or otherwise with the common infectious hepatitis is much disputed; the infection has been transmitted to human volunteers, but not to any other animal species. MacCallum (1944)* has summarized the transmission experiments carried out up to the present.

Rabies: Webster (1939) has introduced improved methods for making and testing the inactivated vaccines used for immunizing dogs. He has also had the temerity (1942) to throw doubts on the efficacy of the time-honoured Pasteurian method of protecting by vaccination a person who has been bitten by a rabid dog; its reputation may, it is suggested, be due to the fact that only a small proportion of bitten persons would develop rabies in any case. With such a terrible disease, deliberate controlled experiments on man are hardly conceivable and one must seek for indirect evidence. If vaccination is as effective as has generally been believed, it is hard to explain why the time elapsing between the bite and the beginning of treatment seems not to affect the result. It would seem advisable to keep an open mind in this matter.

Measles has long baffled research workers because rhesus and other monkeys have been the only susceptible species and even they do not "take" regularly, perhaps because of some chance exposure of some shipments to infection. Several claims to have grown the virus on developing eggs have been made, and those of Rake & Shaffer (1940) seem to be well established. We may, therefore, at last have hopes of progress. After propagation through many generations on chorio-allantoic membranes of eggs, virus has successfully infected both rhesus monkeys and children. Further, the virus has apparently become attenuated so that it has been given to children as a living vaccine by atomizer into a sponge-bag face-mask (Stokes, O'Neil, Shaffer, Rake & Maris, 1943). Modified measles is thus produced in a majority of exposed children, but the reactions are admitted to be by no means negligible. Such children, subsequently exposed to natural measles, are said to have been substantially but by no means entirely protected. This work is in its early stages and will be followed with interest.

Influenza: We have long known (Smith, Andrewes & Laidlaw, 1935) that injection of formolized inactive influenza vaccines will immunize mice and ferrets against influenza, and produce an increase in specific antibodies in man. It has been another matter to prove their efficacy in protecting human beings against infection. One difficulty has been that the duration of increased resistance in man is almost certainly short, so that vaccination, to be effective, must be given shortly before an outbreak; and outbreaks have commonly failed to materialize at expected times. Further, clinical influenza comprises not only infections with the two known, and serologically distinct, viruses A and B, but other conditions of unknown cause giving rise to a similar clinical picture; this makes the evaluation of results a matter of much difficulty. Formolized vaccines have been made recently from embryonic fluids from infected eggs; a high titre of virus can be obtained in this way.

Trials made in the U.S.A. in 1941 gave conflicting results, which indicated at the best an apparent halving of incidence in the vaccinated (Horsfall, Lennette, Rickard & Hirst, 1941). In 1943, tests of vaccine gave decidedly better results (*Commission on Influenza, U.S. Army*, 1944); they were carried out in widely separated areas of the U.S.A., use being made of chick-embryo vaccine concentrated tenfold by adsorption on to chick erythrocytes and elution into a smaller volume of fluid (Francis & Salk, 1942). A reduction of between 3- and 4-fold in incidence was achieved. It may be that the use of better and more concentrated vaccine produced this encouraging result, but one suspects that a fortunate chance played a larger part: an epidemic of influenza A occurred in most of the vaccinated communities two or three weeks after the vaccinations, that is, at the time when protection should be maximal. Profitable future use of a vaccine may depend upon keeping an epidemiological watch on the doings of influenza viruses A and B, so that vaccine may be given at the optimum time and then only. It will certainly be useless to give vaccine to patients who "have influenza every year";

* [see also article by MacCallum and review of relevant papers, *BMB* 177–188]

their infections are most unlikely to be due to either virus. As to pandemic influenza, such as swept the world in 1918–19, nothing but the future can tell us whether such a visitation is due to influenza A or B, to a mutant deriving from either of them, or to an unrelated virus; nor whether vaccines will be of any avail against so virulent a scourge.

REFERENCES

[It is practicable to give only a small number of references as recent virus literature is so extensive. Some of those included are to reviews which should guide the reader who wishes to pursue his enquiries further.]

Andrewes, C. H. (1940) *J. Path. Bact.* **50,** 227
Andrewes, C. H. (1942a) *Brit. J. exp. Path.* **23,** 214
[1] Andrewes, C. H. (1942b) *Proc. roy. Soc. Med.* **36,** 1
Andrewes, C. H. & Glover, R. E. (1941) *Brit. J. exp. Path.* **22,** 91
Andrewes, C. H., King, H., van den Ende, M. & Walker, J. (1944) *Lancet*, **1,** 777
Armstrong, C. (1939) *Publ. Hlth Rep., Wash.* **54,** 1719
Aycock, W. L. (1942) *Medicine*, **21,** 65
Barenberg, L. H., Greene, D., Greenspan, L. & Greenberg, B. (1942) *Aerobiology*, edited by F. R. Moulton, Washington, p. 33
Bedson, S. P. & Bland, J. O. W. (1934) *Brit. J. exp. Path.* **15,** 267
Bryan, W. R. & Beard, J. W. (1940) *J. nat. Cancer Inst.* **1,** 607
Buddingh, G. J. & Polk, A. C. (1939) *J. exp. Med.* **70,** 485
Burnet, F. M. (1933) *J. Path. Bact.* **37,** 107
Burnet, F. M. (1940) *Brit. J. exp. Path.* **21,** 147
Casals, J. (1944) *J. exp. Med.* **79,** 341
Casals, J. & Webster, L. T. (1944) *J. exp. Med.* **79,** 45
Clark, E. & Nagler, F. P. O. (1943) *Aust. J. exp. Biol. med. Sci.* **21,** 103
Commission on Influenza, U.S. Army (1944) *J. Amer. med. Ass.* **124,** 982
Cox, H. R. (1938) *Publ. Hlth Rep., Wash.* **53,** 2241
Dalldorf, G., Douglass, M. & Robinson, H. E. (1938) *J. exp. Med.* **67,** 333
Delbrück, M. & Luria, S. E. (1942) *Arch. Biochem.* **1,** 111
Duffy, C. E. (1944) *Science*, **99,** 517
Edward, D. G. ff. (1941) *Lancet*, **2,** 664
Elford, W. J. (1931) *J. Path. Bact.* **34,** 505
Findlay, G. M. & MacCallum, F. O. (1937a) *Brit. J. exp. Path.* **44,** 405
Findlay, G. M. & MacCallum, F. O. (1937b) *Trans. roy. Soc. trop. Med. Hyg.* **31,** 297
Francis, T. & Salk, J. E. (1942) *Science*, **96,** 499
Gard, S. & Pedersen, K. O. (1941) *Science*, **94,** 493
Goodpasture, E. W. & Anderson, K. (1944) *Amer. J. Path.* **20,** 447
Harris, T. N. & Stokes, J. (1942) *Amer. J. med. Sci.* **204,** 430
Henle, W. & Henle, G. (1943) *Science*, **98,** 87
Henle, W. & Henle, G. (1944) *Amer. J. med. Sci.* **207,** 705
Hirst, G. K. (1941) *Science*, **94,** 22
Hirst, G. K. (1942) *J. exp. Med.* **75,** 49
Hirst, G. K. (1943) *J. exp. Med.* **78,** 99
Horsfall, F. L., Lennette, E. H., Rickard, E. R. & Hirst, G. K. (1941) *Publ. Hlth Rep., Wash.* **56,** 1863
Hoskins, M. (1935) *Amer. J. trop. Med.* **15,** 675
Jungeblut, C. W. & Sanders, M. (1942) *J. exp. Med.* **76,** 127
Kidd, J. G. (1938) *J. exp. Med.* **68,** 703
Kunkel, L. O. (1943) in *Virus Diseases; The Messenger Lectures*, New York
Laidlaw, P. P. (1938) *Virus diseases and viruses; the Rede Lecture*, Cambridge
Laidlaw, P. P. & Dunkin, G. W. (1926) *J. comp. Path.* **39,** 222
Luria, S. E., Delbrück, M. & Anderson, T. F. (1943) *J. Bact* **46,** 57
MacCallum, F. O. (1944) *Proc. roy. Soc. Med.* **37,** 449
MacCallum, F. O. & Findlay, G. M. (1938) *Lancet*, **2,** 136
McClelland, L. & Hare, R. (1941) *Canad. pub. Hlth J.* **32,** 530
Meyer, K. F. (1942) *Medicine*, **21,** 175
Moragues, V., Pinkerton, H. & Greiff, D. (1944) *J. exp. Med.* **79,** 431
Otto, R. & Bickhardt, R. (1941) *Z. Hyg. InfektKr.* **123,** 447
Paul, J. R. (1941) *Medicine*, **20,** 495
Peterson, O. L. (1944) *Proc. Soc. exp. Biol. N.Y.* **55,** 155
Rake, G., Eaton, M. D. & Shaffer, M. F. (1941) *Proc. Soc. exp. Biol. N.Y.* **48,** 528
Rake, G. & Hamre, D. M. (1944) *Proc. Soc. exp. Biol. N.Y.* **55,** 90
Rake, G. & Jones, H. P. (1943) *Proc. Soc. exp. Biol. N.Y.* **53,** 86
Rake, G. & Jones, H. P. (1944) *J. exp. Med.* **79,** 463
Rake, G. & Shaffer, M. F. (1940) *J. Immunol.* **38,** 177
Rake, G., Shaffer, M. F. & Thygeson, P. (1942) *Proc. Soc. exp. Biol. N.Y.* **49,** 545
Rivers, T. M. (1943) in *Virus Diseases, The Messenger Lectures*, New York
Ruska, H. (1943) *Klin. Wschr.* **22,** 703
Shope, R. E. (1941) *J. exp. Med.* **74,** 41
Smith, W., Andrewes, C. H. & Laidlaw, P. P. (1933) *Lancet*, **2,** 66
Smith, W., Andrewes, C. H. & Laidlaw, P. P. (1935) *Brit. J. exp. Path.* **16,** 291
Stanley, W. M. (1935) *Science*, **81,** 644
Stokes, J., O'Neil, G. C., Shaffer, M. F., Rake, G. & Maris, E. P. (1943) *J. Pediat.* **22,** 1 & 17
Taylor, A. R., Sharp, D. G., Bland, D., Beard, J. W., Dingle, J. H. & Feller, A. E. (1943) *J. Immunol.* **47,** 261
Theiler, M. & Gard, S. (1940) *J. exp. Med.* **72,** 49, 69 & 79
Trask, J. D., Paul, J. R. & Melnick, J. L. (1943) *J. exp. Med.* **77,** 531
Webster, L. T. (1939) *J. exp. Med.* **70,** 87
Webster, L. T. (1942) *Rabies*, London & New York
Wells, W. F. & Wells, M. W. (1936) *J. Amer. med. Ass.* **107,** 1698 & 1805
Wells, W. F., Wells, M. W. & Wilder, T. (1942) *Amer. J. Hyg.* **35,** 97
Woodruff, A. M. & Goodpasture, E. W. (1931) *Amer. J. Path.* **7,** 209
Ziegler, J. E. & Horsfall, F. L. (1944) *J. exp. Med.* **79,** 361 & 379
Zinsser, H. & Schoenbach, E. B. (1937) *J. exp. Med.* **66,** 207

[1] [*see BMB* **65**]

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MODERN LABORATORY METHODS IN THE CONTROL OF TYPHOID AND PARATYPHOID-B FEVER

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Two recently introduced laboratory methods have proved to be of great service to the epidemiologist who is called on to investigate outbreaks of enteric fever: (i) typing of typhoid and paratyphoid-B bacilli by means of Vi bacteriophage, and (ii) Vi-agglutination tests as an aid to the detection of chronic typhoid and paratyphoid-B carriers.

Typing of Typhoid Bacilli by the Vi Bacteriophage

Craigie & Yen (1938a, 1938b) made the important discovery that strains of the typhoid bacillus can be divided into a number of well-defined types according to their sensitiveness to type-specific anti-Vi bacteriophages. Until then the typhoid bacillus had been regarded as a single species without varietal subdivision. It was known from the work of Hadley (1925–26), Burnet (1927, 1929) and Levine & Frisch (1934) that the action of bacteriophages on the various Salmonella species is closely related to the presence of certain heat-stable "O" antigens in the bacterial cell.

As soon as the special importance of the so-called "Vi" antigen of the typhoid bacillus was recognized (Felix & Pitt, 1934) a number of investigators, working independently of each other in different countries, established the existence of bacteriophages that were specific for the Vi form of the typhoid bacillus (Sertic & Boulgakov, 1936; Scholtens, 1936; Craigie & Brandon, 1936). These phages attacked typhoid strains irrespective of their origin, provided that the cultures contained an adequate quantity of the Vi antigen.

The great advance due to the work of Craigie & Yen (1938a) was based on their observation of a special adaptability possessed by one particular anti-Vi phage. When this

phage was propagated on typhoid strains isolated from different outbreaks, preparations of bacteriophage were obtained which had developed a high degree of specificity for the particular strain on which they were grown. Strains that were related epidemiologically were invariably found to respond in an identical manner to the different phage preparations, and on this basis Craigie & Yen (1938b) identified a number of distinct Vi-phage types of *Bact. typhosum*. A small proportion of Vi cultures cannot be typed in this way, for reasons that are not yet understood. Such cultures are referred to as " imperfect " Vi forms.

The phage type of a strain is for all practical purposes a permanent character, and typing of the typhoid bacillus by this means gives results as reliable as those obtained in streptococcal or pneumococcal infections by the use of serological tests. It is thus possible to prove or disprove the connection between cases of typhoid fever occurring sporadically or during an outbreak, and between a carrier and the patients for whose infection the former is being held responsible.

It may be stated in passing that the Vi antigen of typhoid strains belonging to different phage types cannot be differentiated by any of the customary serological methods, including cross-agglutination and cross-absorption tests, and phagocytosis and passive-protection tests in mice (Felix & Pitt, unpublished results). It appears, therefore, safe to continue employing a single Vi + O strain of the typhoid bacillus for making typhoid vaccine or therapeutic anti-typhoid serum.

Practical Value of the Typing Method

The observations of Craigie and Yen on the epidemiological significance of Vi-phage types of typhoid bacilli have been amply corroborated. So far reports have been published from Canada (Brandon, 1940; Desranleau, 1942; Crossley, 1942), China (Yen, 1939), the United States of America (Lazarus, 1941; Coleman, 1942a), Great Britain (Felix, 1943; Bradley, 1943) and the Middle East (Boyd, 1943). The authors all agree that the bacteriophage test is a reliable guide to the epidemiological investigation of outbreaks or sporadic cases of typhoid fever.

In Britain the new method has been employed extensively since 1940. The Emergency Public Health Laboratory Service established a reference laboratory to which cultures of *Bact. typhosum* isolated in public health or clinical laboratories in any part of Britain have been submitted for typing by the bacteriophage technique. The application of the test has on many occasions proved to be of the greatest value to those concerned with the field investigations. In fact, the information derived from the typing of strains at a central reference laboratory often provided the only link between a number of widely scattered cases, and thus led to the detection of the responsible chronic carrier. The fascinating story of one of these investigations has been published by Bradley (1943). In this instance 23 apparently sporadic cases occurring in four counties and ten administrative districts, during the course of two years, were traced to a persistent carrier on a farm 100 miles away. Other striking examples of the value of the new typing method were encountered in localities varying from small rural districts to the metropolitan boroughs of London. These experiences led to the conclusion that phage typing is an indispensable aid to successful epidemiological field-work (Felix, 1943).

The standard phage preparations and standard type-cultures originally recommended by Craigie represented 18 Vi-types and subtypes. Subsequently a revised and simplified typing scheme was suggested by Craigie (1942), but his investigations regarding the required phage preparations have not yet been completed. Four additional Vi-phage types were identified in Britain (Felix, 1943); three of these are indigenous and the fourth may have been introduced from abroad. It is obvious that workers in different countries who are employing the bacteriophage typing method should maintain close contact and exchange cultures and phage preparations before types or subtypes are accepted as new and added to the original scheme of Craigie and Yen.

The following example illustrates the value of this kind of co-operation. In a small outbreak of typhoid fever in Britain, the cultures isolated from all the cases and from the responsible carrier were found to belong to a hitherto unknown Vi-phage type of the typhoid bacillus. The strain was provisionally labelled Type T. It was subsequently learnt that the carrier had acquired the infection during the war in South Africa more than forty years ago, and it appeared probable that the strain was indigenous to South Africa. Cultures of this strain and the corresponding specific Vi-type phage were sent to Professor A. Pijper of the University of Pretoria; and Miss C. G. Crocker, who has had considerable experience of the phage-typing technique, soon reported that typhoid strains isolated early in 1944 from two patients in the Johannesburg district and from a patient in the Pretoria General Hospital belonged to Type T.

Typing of Paratyphoid-B Bacilli with the Vi Bacteriophage

In many countries, including Britain, paratyphoid-B fever is more prevalent than typhoid fever. It appeared, therefore, highly desirable to investigate the possibility of applying Vi-bacteriophage action to the examination of paratyphoid-B cases and carriers.

The presence in cultures of *Bact. paratyphosum* B of an antigen resembling the typhoid Vi antigen was described some years ago (Felix & Pitt, 1936), but most workers paid little attention to this finding. Later experiments carried out by Felix and Pitt before the war (but not yet published) strongly indicated that paratyphoid-B bacilli possess a heat-labile somatic antigen that is essentially similar to the typhoid Vi antigen, though differing from it in certain respects. This fact provided the theoretical basis of the investigations carried out in the Emergency Public Health Laboratory Service during 1941–43, which led to the recognition of anti-O and anti-Vi bacteriophages acting on *Bact. paratyphosum* B, and thus made it possible to apply the bacteriophage technique to the typing of paratyphoid-B bacilli (Felix & Callow, 1943).

Anti-O phages are very common and are readily grown from faeces, but they are non-specific. These phages attack not only paratyphoid-B bacilli but also many other Salmonella species which share the same O-antigenic components. On the other hand, the specific anti-Vi phages are rarely found in faeces, but they can be isolated from " lysogenic " rough variants or from phage-contaminated smooth cultures of the paratyphoid-B bacillus. Anti-Vi phages can be induced to develop a high degree of specificity for particular paratyphoid-B strains, whereas anti-O phages are incapable of such adaptation. By the use of four different preparations of adapted BVi phage, Felix and Callow evolved a typing scheme of paratyphoid-B bacilli which is almost identical with that which was first devised by Craigie and Yen for the typing of typhoid bacilli.

Only a small number (7 %) of 714 strains isolated in all parts of Britain could not be typed by the four Vi-type phages originally used by Felix and Callow. In this respect the results with paratyphoid-B cultures were even more satisfactory than with typhoid cultures, for, in a series of 432 typhoid Vi strains, 15·9 % were untypable with the 22 different Vi-phages employed. The number of known Vi-types and subtypes of the paratyphoid-B bacillus indigenous to Britain is now 5. It is, however, becoming apparent that hitherto unknown phage types are being introduced from abroad.

The epidemiological significance of paratyphoid-B Vi-phage types is the same as that of typhoid Vi-phage types. The first field inquiry, based on the results of the phage typing, into an outbreak of paratyphoid-B fever in a mixed urban and rural area was conducted during 1941 by the Ministry of Health in co-operation with the Emergency Public Health Laboratory Service, and clearly showed that there was complete harmony between the laboratory and the field findings (Hutchinson, 1943). A remarkable performance of the typing method was recently described by King (1944). It is the story of a symptomless carrier of paratyphoid-B bacilli of Type 3a, who had disappeared during an outbreak in 1941 but was re-discovered among the employees of a factory two years later when the sewage effluent was being examined for the presence of typhoid bacilli.

Detection of Chronic Typhoid and Paratyphoid-B Carriers with the aid of Vi-Agglutination Tests

i. *Chronic typhoid carriers:* The detection of a chronic carrier is usually a difficult task, though the methods of isolating typhoid and paratyphoid bacilli have been greatly improved through the introduction of refined culture media. The main difficulty is due to the fact that excretion is frequently intermittent, so that repeated examination of the excreta over a long period of time may be necessary.

Most workers now employ the test for the presence of typhoid Vi agglutinins as a preliminary screen for a suspected carrier. Since the test was first suggested by Felix, Krikorian & Reitler (1935) it has been used extensively in Britain (Felix, 1938a), South Africa (Pijper & Crocker, 1937a; 1937b; 1943), the United States of America (Eliot, 1940; Eliot & Cameron, 1941; Coleman, 1942b; Klein, 1943; Schlesinger, 1943), Italy (Giovanardi, 1936; 1937) and India (Bhatnagar, 1938; Bensted, 1940). These authors all agree on the usefulness of the test. Less favourable results were reported from the Sudan (Horgan & Drysdale, 1940) and Southern Rhodesia (Davis, 1940; Radovsky, 1942).

The practical value of the Vi-agglutination test is greatly enhanced by the fact that the reaction is independent of the intermittency in the excretion of bacilli. A few examples published recently may be quoted here. Schlesinger (1943) recorded the case of a woman from whom no fewer than 18 faecal specimens were negative, but continued search was encouraged by the presence of Vi agglutinins, and typhoid bacilli were isolated from bile and faeces after the injection of magnesium sulphate through a duodenal tube. Pijper & Crocker (1943) gave the history of a positive Vi-reactor in whose faeces typhoid bacilli were never demonstrated, though they were constantly present in discharges from a biliary fistula. Fry (1944) examined specimens of faeces from an intermittent excretor at weekly intervals over a period of 16 months. The specimens were examined on Wilson-Blair plates, and by subcultures on to Wilson-Blair medium after enrichment in both Müller's and Kauffmann's tetrathionate broth. Yet, of 80 specimens, only 22 were positive for *Bact. typhosum*. The longest negative interval was 9 successive weeks; if no enrichment media had been used, the longest negative interval would have been 27 weeks. Four tests for Vi agglutination which were carried out during this period gave the same result on each occasion, namely, a positive reaction at 1 in 20. At the post-mortem examination *Bact. typhosum* was isolated from the contents of the duodenum.

The technique of the Vi-agglutination test has been much simplified since preserved suspensions were adopted instead of living cultures (Felix, 1938b), and especially since the introduction of Bhatnagar's strain Vi I, which is a pure reagent for the demonstration of typhoid Vi agglutinins (Bhatnagar, Speechly & Singh, 1938). In Britain a standard agglutinable TVi suspension and a standard agglutinating TVi serum are available through the Standards Laboratory (*Medical Research Council*), Oxford, and are strongly recommended for general use.

The frequency of a positive Vi reaction in chronic typhoid carriers is remarkably high. From my experience over a period of nearly ten years it would appear that not more than 5% to 10% of true chronic carriers will give a negative Vi reaction. When the carrier condition has lasted for a very long time, for example, periods of 30 or 40 or more years, the power of producing Vi antibody often tends to be exhausted.

The Vi test can also be used for the detection among recovered typhoid patients of those who are likely to become chronic carriers. The customary three negative examinations of the excreta are no guarantee that potential chronic carriers are not discharged from hospital. Such carriers can be detected by two tests for Vi agglutination, the first carried out on the eve of discharge from hospital, the second after an interval of about six weeks. A steady or rising Vi-titre will arouse suspicion as to a possible chronic carrier condition, a decreasing Vi-titre or a negative Vi reaction will indicate freedom from infection. The follow-up of recovered patients in this way seems to make it possible in time to eradicate typhoid fever completely.

ii. *Chronic paratyphoid-B carriers:* It is generally believed that the Vi-agglutination test is applicable only to typhoid and not to paratyphoid carriers. Unpublished investigations carried out by the writer in the Emergency Public Health Laboratory Service during the present war have shown that the method can be applied successfully to the examination of paratyphoid-B carriers as well. The Vi test, however, is more complicated in paratyphoid-B than in typhoid, since a "pure" Vi variant of *Bact. paratyphosum* B, comparable to Bhatnagar's typhoid strain Vi I, has not yet been isolated. When a serum is tested for Vi antibody to paratyphoid-B bacilli, its O-antibody content must, therefore, first be completely removed by absorption with a pure O culture. Most of the chronic paratyphoid-B carriers that have been examined so far gave a positive BVi reaction, but the frequency of a positive reaction in paratyphoid B is not as high as that of a positive TVi reaction in typhoid carriers.

The proportion of mild and symptomless cases is very much greater in paratyphoid B than in typhoid fever, and one of the difficulties that often arises in the course of an epidemiological investigation is the question whether a person who has been found excreting paratyphoid-B bacilli is a true chronic carrier or a temporary excretor following a mild or symptomless infection. The former may be the original source of the outbreak, whereas the latter is merely one of its victims, though he, or she, may have been responsible for the spread of the infection. In all the recent outbreaks of paratyphoid-B fever in Britain, the persons identified as responsible for the spread of infection were later proved to be temporary excretors. The true culprits, namely the chronic carriers, escaped detection. In the diagnosis of the true carrier the final verdict depends on the result of the bacteriological examination of the excreta, which must be continued for not less than a year, but useful information is often obtained much sooner from two or three examinations of the blood serum at intervals of about six weeks. A decreasing BVi titre will indicate temporary excretion, whereas a steady or rising titre will arouse the suspicion that the person has been or is likely to become a chronic carrier of the infection.

References

Bensted, H. J. (1940) *J. Roy. Army Med. Cps*, **74**, 19
Bhatnagar, S. S. (1938) *Brit. med. J.* **2**, 1195
Bhatnagar, S. S., Speechly, C. G. J. & Singh, M. (1938) *J. Hyg., Camb.* **38**, 663
Boyd, J. S. K. (1943) *Brit. med. J.* **1**, 719
[1] Bradley, W. H. (1943) *Brit. med. J.* **1**, 438
Brandon, K. F. (1940) *Canad. Publ. Hlth J.* **31**, 10
Burnet, F. M. (1927) *Brit. J. exp. Path.* **8**, 121
Burnet, F. M. (1929) *J. Path. Bact.* **32**, 15
Coleman, M. B. (1942a) *Ann. Rep. Div. Lab. Res., Albany*, p. 52
Coleman, M. B. (1942b) *Amer. J. pub. Hlth*, **32**, 843
Craigie, J. (1942) *Canad. publ. Hlth J.* **33**, 41
Craigie, J. & Brandon, K. F. (1936) *J. Path. Bact.* **43**, 233
Craigie, J. & Yen, C. H. (1938a) *Canad. publ. Hlth J.* **29**, 448
Craigie, J. & Yen, C. H. (1938b) *Canad. publ. Hlth J.* **29**, 484
Crossley, V. M. (1942) *Canad. publ. Hlth J.* **33**, 337
Davis, L. J. (1940) *J. Hyg., Camb.* **40**, 406
Desranleau, J. M. (1942) *Canad. publ. Hlth J.* **33**, 122
Eliot, C. P. (1940) *Amer. J. Hyg.* **31**, B, 8
Eliot, C. P. & Cameron, W. R. (1941) *Amer. J. publ. Hlth*, **31**, 599
Felix, A. (1938a) *Lancet*, **2**, 738
Felix, A. (1938b) *J. Hyg., Camb.* **38**, 750
[2] Felix, A. (1943) *Brit. med. J.* **1**, 435
[3] Felix, A. & Callow, B. R. (1943) *Brit. med. J.* **2**, 127
Felix, A., Krikorian, K. S. & Reitler, R. (1935) *J. Hyg., Camb.* **35**, 421
Felix, A. & Pitt, R. M. (1934) *Lancet*, **2**, 186
Felix, A. & Pitt, R. M. (1936) *Brit. J. exp. Path.* **17**, 81
Fry, R. M. (1944) *Mon. Bull. Min. Hlth Emerg. publ. Hlth Lab. Serv.* **3**, 103
Giovanardi, A. (1936) *Boll. Soc. ital. Biol. sper.* **11**, 163 & 943
Giovanardi, A. (1937) *Z. Hyg. InfektKr.* **120**, 273
Hadley, P. (1925–26) *Proc. Soc. exp. Biol. N.Y.* **23**, 443
Horgan, E. S. & Drysdale, A. (1940) *Lancet*, **1**, 1084
[4] Hutchinson, J. R. (1943) *Brit. med. J.* **2**, 130
King, G. J. G. (1944) *Mon. Bull. Min. Hlth Emerg. publ. Hlth Lab. Serv.* **3**, 84
Klein, M. (1943) *J. infect. Dis.* **72**, 49
Lazarus, A. S. (1941) *Amer. J. publ. Hlth*, **31**, 60
Levine, P. & Frisch, A. W. (1934) *J. exp. Med.* **59**, 213
Pijper, A. & Crocker, C. G. (1937a) *J. Hyg., Camb.* **37**, 332
Pijper, A. & Crocker, C. G. (1937b) *S. Afr. med. J.* **11**, 252
Pijper, A. & Crocker, C. G. (1943) *J. Hyg., Camb.* **43**, 201
Radowsky, H. (1942) *Trans. roy. Soc. trop. Med. Hyg.* **36**, 45
Schlesinger, E. R. (1943) *Amer. J. publ. Hlth*, **33**, 1257
Scholtens, R. T. (1936) *J. Hyg., Camb.* **36**, 452
Sertic, V. & Boulgakov, N. A. (1936) *C. R. Soc. Biol. Paris*, **122**, 35
Yen, C. H. (1939) *Proc. Soc. exp. Biol. N.Y.* **41**, 162

[1] [*see BMB* **70**]
[2] [*see BMB* **69**]
[3] [*see BMB* **149**]
[4] [*see BMB* **150**]

HOSPITAL INFECTION: A HISTORICAL REVIEW

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Any congregation of people increases the chances for the spread of infection. Thus it is that towns, gaols, ships, barracks, schools, and even hospitals, have from time immemorial been the breeding ground of epidemics, or as Greenwood (1935) has aptly called them, "crowd diseases". That hospitals designed for healing the sick should, from their very nature, carry some added danger to those who require their healing aid has long worried the hospital administrator. As that greatest of hospital reformers, Florence Nightingale, has said, "it may seem a strange principle to enunciate that the very first requirement in a hospital is that it should do the sick no harm". To-day the problem of hospital infection, although less serious and different in kind from that experienced before the Listerian era, still remains unsolved and, in recent years, has stimulated much research directed towards its control and elimination. In the past century British workers have played a large part in unravelling the intricacies of this complex problem.

Hospital Infection in the 19th Century

Before Lister demonstrated the part that bacteria play in the production of wound-infection, the surgical wards of most hospitals were filled with patients whose wounds, whether accidental or intentional, almost always became septic, so that healing, when it occurred, was by granulation or scabbing. Unfortunately the sepsis spread beyond the local wound in many cases, and more generalized infections, such as erysipelas and cellulitis, pyaemia, septicaemia and hospital gangrene, were common complications of any wound. The first three of these generalized infections would be recognized to-day as streptococcal or staphylococcal in aetiology: the bacteriology of hospital gangrene—described by Cameron [1] (1907) as a progressive mortification of the tissues adjacent to the wound and apparently infectious—cannot be established, as it is doubtful whether anything comparable to it now exists. The result of the gross wound-sepsis was a high morbidity- and mortality-rate among surgical patients; for example, practically all cases of compound fracture became infected and 25% to 50% of them died. Because the constitutional disturbance accompanying the wound infection affected the patient's appetite, a light diet, low in calories, was always prescribed. This light hospital diet was continued as a routine long after the need for it ceased to exist, and only to-day is it being realized that "hospital diet" is quite inadequate for the needs of most patients. The wards themselves had constantly a characteristic sickly odour, and surgery was confined to a limited field—about one-fifth of the operations were amputations—largely because surgeons feared for the outcome of more venturesome interference. The surgical wards of most hospitals acquired an evil reputation, and as the results of operative procedures were so much better when carried out in private houses, many doctors preferred to bring the surgeon to the patient rather than the reverse. Even as late as 30 years ago, I remember as a boy how in country districts the surgeon frequently came to operate, often under great difficulties, in a private house because of the countryman's fear of going to the infirmary. No wonder that certain hospital authorities felt impelled to destroy the hospitals and build anew.

If surgical wards had the worst reputation they were not alone in fostering secondary infection. In a careful and penetrating analysis of mortality-rates in maternity hospitals, Florence Nightingale showed that many of these institutions had bad records of puerperal sepsis with "blood-poisoning" as a frequent complication, and with maternal mortality-rates as much as 7 times as high as those occurring in domiciliary practice. The effect of overcrowding, of large undivided wards and poor ventilation, in encouraging outbreaks of puerperal sepsis in these institutions was recognized by her, and the infectiousness of the condition was admitted, although she was apparently unaware of the earlier researches of Semmelweis on the control of the manual transference of infection by hand-washing in "chlorine-water".

Earlier, Miss Nightingale, as a result of her unique experience in the Crimean War, where, incidentally, 2 out of every 5 patients admitted to the large general hospital at Scutari died, had drawn attention in forceful language to the unsatisfactory design and management of hospitals, and had indicted agglomeration of a large number of sick under one roof, deficiency of space, deficiency of ventilation, and deficiency of light, as the principal factors predisposing to hospital infection and the unsatisfactory results of hospital treatment. In children's hospitals, conditions were even worse. Throughout Europe, till late in the 19th century, mortality-rates in children's hospitals were excessively high, and parents feared to send their children to these institutions, "where they died not from the malady for which they entered the hospital, but from that which they contracted therein". Thus hospital infection played havoc among medical as well as surgical patients. No doubt also the great increase in urban populations associated with the industrial revolution in the 19th century helped to overcrowd hospitals that were very inadequately staffed and equipped, and we know to-day these are among the most important factors contributing to cross-infection in hospital.

One cannot close this grim chapter without paying a tribute to Florence Nightingale who in three slim volumes *Notes on hospitals* (1859), *Notes on nursing* (1860), and *On lying-in institutions* (1871) had shown how well she comprehended the root causes of hospital infection, and had enunciated the principles of good hospital design and management, and what is even more important, of good nursing. It is particularly interesting at the present time to note that she was a strong advocate of damp-dusting and -sweeping for getting rid of dust, for as she said "air can be soiled just like water".

Listerian Principles

Pasteur's demonstration of the bacterial origin of fermentation was the stimulus which inspired Lister (see Godlee, 1917) to devise methods for preventing infection of wounds in his surgical wards. Believing that bacteria causing "putrefaction," or sepsis as we should now call it, came from a variety of sources outside the body (and at that time he naturally thought that any extraneous micro-organism was pyogenic), Lister introduced antiseptics to prevent access of these bacteria from hands, instruments, dust, air, etc., to open wounds—e.g. compound fractures, incised abscesses which in those days were mostly tuberculous, and intentional wounds. Carbolic acid had been used as a disinfectant for sewage,[1] and Lister employed it in a variety of forms: first as the crude undiluted fluid; as a 1:20 solution of phenol crystals; as a 1:5 solution in oil, made into a putty: as a mixture of phenol in shellac spread on calico; and finally as an antiseptic gauze.

Later, probably impressed by Tyndall's (1881) work on the pollution of the atmosphere with particulate matter, Lister advocated the use of the carbolic spray for preventing access of air-borne bacteria to the wound, but after some years' experience of this method he became persuaded of its uselessness, since bacteria in the atmosphere seemed to have little pathogenicity. Lister knew that carbolic acid was harmful to tissues, but its caustic action seemed a low price to pay for preventing infection and saving many lives from the terrors of septicæmia and hospital gangrene. It is perhaps apposite to remark here that proflavine, one of the most useful of modern antiseptics for the treatment of war wounds, is also a tissue poison. The important point to note in Lister's work is that for the first time *an effective barrier had been imposed between infecting organism and susceptible tissue.*

[1] [Sir Hector Cameron was the pupil and personal friend of Lord Lister and in his small book *On the evolution of wound treatment during the last forty years* he traces the development of modern surgery from Lister's antiseptic methods.]

[1] [Lemaire's use of carbolic acid in France was not known to Lister until 1867.]

There were many contemporary critics of Lister's antiseptic surgery, but the criticisms were mainly of the methods employed, and not of the principles which constituted the first premeditated step in the control of hospital infection. The dramatic results can best be recorded in Lister's own words in 1867:

"But since the antiseptic treatment has been brought into full operation, and wounds and abscesses no longer poison the atmosphere with putrid exhalations, my wards, though in other respects under precisely the same circumstances as before, have completely changed their character; so that during the last nine months not a single instance of pyaemia, hospital gangrene, or erysipelas has occurred in them.

"As there appears to be no doubt regarding the cause of this change, the importance of the fact can hardly be exaggerated."

And in a letter to his father in the same year he remarked:

"I now perform an operation for the removal of a tumour, etc., with a totally different feeling from what I used to have; in fact, surgery is becoming a different thing altogether."

From antiseptic surgery there gradually evolved the elaborate ritual of aseptic surgery dependent mainly on sterilization by heat. While sufficiently adequate for the deliberate surgery of peace-time, the aseptic technique, as we shall see later, may fail in the face of the more exacting conditions of war.

Cross-infection in Paediatric and Isolation Hospitals

As the beneficial effects of antiseptic surgery gradually became accepted, those in charge of medical patients, and in particular of children's hospitals, began to realize that there was an urgent need for medical as well as surgical antisepsis. The criticisms of Florence Nightingale had already done much to restrict overcrowding and improve ventilation, but mortality rates in children's hospitals were still, in the latter half of last century, 20% to 40% of all admissions. However, the decade between 1880 and 1890 saw bacteriology established as a new science, the infectivity of many diseases was becoming accepted, and experiments were already being made on the mode of transference of the infectious agents. The first specific measures aimed at reducing cross-infection in medical wards were carried out by Grancher and Hutinel around 1890 (Grancher, 1888; Hutinel, 1894) in two children's hospitals in Paris. In these hospitals, patients with diphtheria, scarlet fever and measles were being nursed beside non-infective patients, and the case-mortality of, for example, measles ranged from 40% to 50%. Among the measures introduced by Hutinel at the *Hospice des Enfants Assistés* were: disinfection of linen; thorough cleansing of wards every two weeks; examination of all new admissions for sore throat, etc.; strict isolation of cases of diphtheria and other infectious diseases; subdivision of large wards into "boxes" for one or two beds where patients with bronchopneumonia, peritonitis, diarrhoea, syphilis, etc., could be isolated; use of holland overalls by nurses and doctors; and washing of hands with antiseptics.

A little later the work of Flügge and his pupils (1897, 1899) showing that infective droplets expelled by tuberculous patients during coughing had a limited range of about 3 feet [about 1 m.], and that guinea-pigs could be infected much more easily by direct exposure to a tuberculous patient than by inhalation of dried infected sputum, led to the doctrine of the spray or droplet spread of respiratory diseases with its implication of transference only by close contact, instead of an aerial spread, for which Power (1880–81) from his studies on the epidemiology of smallpox had been such a strong advocate. Thus, instead of complete wooden barriers, Grancher later used screens around individual patients. Everything used by each patient was kept separate, and crockery, etc. was always boiled after use. As a result of this technique he claimed that secondary cases of measles were reduced by two-thirds, while those of scarlet fever and diphtheria were reduced from about 24 to nil per annum. The system of cubicle-isolation with incomplete partitions was introduced into Britain in 1906 by Pugh, who found that only measles and chicken-pox among the specific fevers were resistant to this method of controlling hospital infection. Instead of visible barriers, such as partitions or screens, Crookshank (1910) introduced a technique for nursing infectious patients by "barrier" for single patients and "bed-isolation" for all patients in the ward. Essentially this technique involved strict precautionary measures by trained nursing staff to prevent the spread, manually or by ward equipment, of infectious material from the isolated patient to his neighbours. The bed-isolation technique was put to the test in two small wards by Rundle & Burton (1912) who, over a period of 2 years, nursed 473 patients suffering from a variety of diseases (puerperal fever, erysipelas, pertussis, chickenpox, rubella, doubtful scarlet fever and diphtheria, convalescent measles) with only two secondary cases of scarlet fever. Thus the success of the bed-isolation technique supported Flügge's teaching and demonstrated the important part which the hands may play in the transference of infection (see Harries, 1935). Meanwhile the advent of the 1914–18 war was to upset the complacency of surgeons who had come to believe that aseptic surgery was sufficient to control the spread of infection in surgical wards.

Hospital Infection of Open Wounds

Although in the 1914-18 war the incidence of obvious infection was, apart from gas gangrene, insignificant among wounded soldiers on admission to hospital, surgeons soon became depressed at the high proportion of wounds which subsequently became septic or were complicated by spreading cellulitis, osteomyelitis, pyaemia and septicaemia. It was almost akin to pre-Listerian days, and the aseptic technique seemed powerless to prevent this gross wound sepsis. Bacteriological examination showed that less than 20% of the wounds yielded *Strept. pyogenes* or *Staph. aureus* on arrival at the casualty clearing station or base hospital, whereas some 80% to 90% were infected with the streptococcus 2 weeks after admission to hospital (Stokes & Tytler, 1918; Fleming & Porteous, 1919). The trouble was, of course, that war wounds could not be closed by primary suture, and there was therefore a great agglomeration of patients with open wounds, susceptible to infection and frequently exposed to the risk during the daily dressing. The obviously infected cases with purulent discharge or persistent sinuses remained for weeks or months in the wards and constituted a heavy load of infection from which newly-admitted patients could scarcely avoid becoming in turn infected.

There was a renewal of interest in antiseptic surgery and a great stimulus to the production of new antiseptics. Outstanding among these were: the aminoacridine compounds or "flavines" introduced for this purpose by Browning and his colleagues (see Browning, 1943) which had the advantage over older antiseptics of having little toxicity for tissues and being unaffected by mixture with serum; and the hypochlorites, e.g. eusol (Smith, Drennan, Rettie & Campbell, 1915; Smith, Ritchie & Rettie, 1917), with their cleansing effect on discharging surfaces, although they rapidly became inert and had to be frequently renewed, as in the Carrel-Dakin method of irrigation. If these new antiseptics had considerable advantages over Lister's carbolic acid, the Listerian principle of *preventing* access of pathogenic bacteria to the wound was often forgotten, and antiseptics were usually applied after sepsis had already declared itself clinically. Pus interferes with the action of most antiseptics, and the views of surgeons about the efficacy of local antisepsis were by no means unanimously favourable. With the return of peace-time surgery, the need for antiseptic dressings was greatly reduced, and although eusol and the flavines still enjoyed a certain popularity the stimulus of necessity for the discovery of new and better antiseptics was lacking.[1]

Although surgeons had gone back with a sigh of relief to aseptic surgery, those responsible for the treatment of patients with burns or with disease of the ear, nose and throat could not fail to be impressed by the frequency of infection among such patients. For example, pyrexia commencing on the second or third day and continuing for a week was a common feature in burns of the second or third degree, while "clean" operations, e.g. for cleft palate in otorhinological wards, frequently failed to achieve the desired result because of secondary infection.

An investigation of the bacteriology of burns (Cruickshank, 1935) showed that while 11% of newly-admitted patients

[1] [The status and relative merits of local antiseptics at the beginning of the present war has been well summarized by Garrod in the *Lancet's* "*War primer on wound infection*" (1940). See also *BMB* 48, a review (1943) of recent advances in the antiseptic treatment of wounds, by the same author.]

yielded scanty haemolytic streptococci from the burnt surface, within 3 to 6 days 66% were grossly infected with the haemolytic streptococcus, while 2 out of every 100 patients developed scarlet fever, and sore throats and septic fingers were unduly prevalent among the nursing staff. Haemolytic streptococci were found in considerable numbers in the air and dust of the burns wards, but rarely in general surgical wards and not at all in medical wards. In the previous year Griffith (1934) had, after much painstaking research, published his classical work on the serological division into a large number of types of the haemolytic streptococci responsible for human infections. The application of his technique to the burns streptococci showed that one particular type of streptococcus was present in a number of infected burns in the same ward. This was the first piece of circumstantial evidence indicating that spread of the haemolytic streptococcus from infected to uninfected wounds was occurring, and that infected dust was a possible mode of transference of the organism. In 1936, Okell & Elliott reported 14 outbreaks of streptococcal cross-infection during 3½ years in 4 small otorhinological wards, the secondary infection varying from pharyngitis, otitis, mastoiditis, adenitis, to erysipelas, scarlet fever, bronchopneumonia and septicaemia. The adoption of a modified bed-isolation technique carried out with care and intelligence by an instructed nursing and medical staff considerably reduced the incidence of cross-infection in their wards.

In the present war, the occurrence of secondary hospital infection of open wounds has been amply confirmed by Miles and his colleagues (1940), using repeated swabbings and serological typing of the streptococcus as detective devices. The conditions of war make it very difficult to avoid infection of wounds, and, despite the introduction of a "no-touch technique," and the prophylactic use of sulphonamides, both locally and by mouth, war-wound sepsis is still a great menace. Antiseptics have come into fashion again, and each new preparation is hailed as the answer to the surgeon's prayer. Among antiseptics that have proved their worth in controlling wound sepsis in recent years are 1% proflavine powder (Mitchell & Buttle, 1942); a sulphathiazole-proflavine powder in the proportion of 99 : 1 and a total dosage of 10–15 g. (McIntosh & Selbie, 1944); propamidine [1]; and, of course, penicillin in solution, powder or cream.[2] However, it must not be forgotten that radical surgery with thorough excision of any lacerated wound or necrotic tissue is a prime necessity in dealing with war wounds, while sufficient emphasis is still not given to the Listerian principle of prophylactic antisepsis.

Air-Borne Infection [3]

Although Flügge's doctrine that most respiratory diseases are conveyed by spray or droplet, requiring close contact between infected and susceptible persons, has long been generally accepted by clinicians, the possibility of the transmission of infection by air is no new conception. Apart from theories about miasma, it is nearly half a century since Washbourn & Eyre (1902) showed that living pneumococci could be isolated from the dust of ward and laboratory, and about the same time German workers carried out a large series of tests demonstrating the prolonged viability of naturally dried pathogens, such as the diphtheria and tubercle bacilli, the streptococcus and staphylococcus. However, despite the evidence (see Thomson, 1916) that the infecting agents of chickenpox and measles could be carried considerable distances, it is only in recent years that the importance of "air-borne" infection has again come into prominence.

Droplet-nuclei. Renewed interest in the possibility of an aerial spread of infection was stimulated by the work in America of Wells and his associates (see Wells & Wells, 1936), who showed that droplets expelled during talking, coughing and sneezing do not *all* fall to the ground within a few feet of the individual, but that instead, droplets of less than 0·1 mm. diameter, called by Wells droplet-nuclei, are so light, or become so by evaporation, that they remain suspended in the air like particles of smoke and may be carried considerable distances by air-currents.

Precise data on the fate of expelled droplets have recently been recorded by Bourdillon & Lidwell (1941) using flash photography. When a patient with a respiratory infection coughs or sneezes, the majority of the droplets describe a curved trajectory to the ground within a radius of 4 to 6 feet [about 1–2 m.] (see also Hare, 1940), so that a susceptible person would have to stand in very close proximity to be directly infected by these coarser droplets. The smallest droplets remain suspended in the air for periods of 30 minutes or more, and although dispersion of these droplet-nuclei by air-currents must mean a great dilution of the *materies morbi*, epidemiological evidence indicates that in many virus diseases the infecting dose is very small. Thus an aerial carriage of droplet-nuclei would help to explain the high infectivity of such infections as smallpox, chicken-pox, measles, and dog distemper (see Dunkin & Laidlaw, 1926). Andrewes & Glover (1941) showed that influenza virus could spread from one ferret-cage to another along an S- or U-shaped duct, allowing the passage only of droplet-nuclei or very fine dust.

Dust-borne infection. Meanwhile, the larger expelled droplets have fallen to the ground or on to bed-clothes and become dried. If they contain bacteria that withstand natural drying, as is the case with many respiratory pathogens, they infect the dust on the floor and the fluff of the bed-clothes. Ward activities, in particular sweeping, dusting and bed-making, raise these infective particles into the air, so that during bed-making in a scarlet-fever ward a patient may inhale 100 to 200 dried haemolytic streptococci. Yet clinicians have been unwilling to believe that these dried and possibly "emasculated" bacteria could overcome the natural resistance of tissues and initiate infection. However, strong circumstantial evidence in favour of dust-borne infection has been provided by:

i. The occurrence of tonsillitis due to a particular type of streptococcus in an individual who swept out a cubicle vacated 3 days earlier by a puerperal patient infected with the same serological type of streptococcus (White, 1936).

ii. Two small outbreaks of puerperal sepsis where direct transference by human carriers seemed to be excluded (Cruickshank & Godber 1939).

iii. The occurrence of an explosive outbreak of streptococcal infection following influenza (Cruickshank & Muir 1940).

iv. The control of secondary streptococcal infection among children in a measles ward by preventing dissemination of infected dust (Wright, Cruickshank & Gunn, 1944).

Additional supportive evidence was the finding by Brown & Allison (1937) of haemolytic streptococci in considerable numbers in the air of scarlet-fever wards of the same serological types as were found in the upper air passages of the patients. These two workers had earlier (1937) shown that most of the complications of scarlet fever in hospital patients were due to cross-infection with serological types of the streptococcus other than that causing the original disease. Their findings were fully confirmed by de Waal (1940). The demonstration by Anderson, McLeod and colleagues (1931, 1933) of three biological types of the diphtheria bacillus called by them *gravis*, *intermedius* and *mitis*, afforded them the opportunity to prove that cross-infection was a frequent, if clinically insignificant, occurrence in diphtheria wards. Later Crosbie & Wright (1941) and Wright, Shone & Tucker (1941) showed that infected dust was almost certainly one of the vehicles which facilitated diphtheritic cross-infection. Among virus diseases, psittacosis has been acquired by attendants from the sweepings of parrot cages (McCoy, 1934), while evidence of air- and dust-borne rickettsial infection, e.g. typhus (van den Ende, Harries, Stuart-Harris, Steigman & Cruickshank, 1943) and Q fever (Hornibrook, Nelson, Dyer, Topping & Bengtson, 1940) has also recently been recorded.

Methods of control. The methods—chemical and physical disinfection of the air, dust-suppressive measures—for dealing with air-borne infection are discussed by Professor Miles on another page.[1] All that need be said here is that much work has been done in Britain in the development of these methods. Thus, Douglas, Hill & Smith used hypochlorite sprays for

[1] [*see BMB* 48 & 58] [2] [*see BMB* 197–238]

[3] ["Air-borne infection" is used here to mean the transference of infection through the air other than by direct "droplet" infection which requires close contact between infector and infected (Cruickshank, 1940).]

[1] [*see BMB* 554]

aerial disinfection as early as 1928, and Masterman (1938) developed the technique and enunciated the principles of disinfection by hypochlorite, which he maintained acted in virtue of the formation of hypochlorous acid gas. His work has been confirmed by Edward & Lidwell (1943) and the principle of aerial disinfection by vaporization of suitable substances which saturate the air and condense on bacteria-carrying particles has now become generally accepted (see Robertson, 1942). A great volume of research, much of it unpublished, on the problems of aerial disinfection has been carried out by teams of workers at the National Institute for Medical Research, London. Some of their earlier findings have been summarized by Andrewes (1940). Twort and his colleagues at Portslade (Twort, Baker, Finn & Powell, 1940; Baker & Twort, 1941; Twort & Baker, 1942) have also made valuable contributions to the subject.

Dust-suppressive measures have been developed by van den Ende and his colleagues (1940, 1941a, 1941b) by the utilization of certain oils, e.g. crude paraffin oil and "technical white oil," for application to floors and to bed-linen. The value of such measures has been well demonstrated by the reduction of secondary bacterial infection in measles wards (Wright, Cruickshank & Gunn, 1944), of cross-infection in scarlet-fever wards (unpublished observations) and of upper respiratory infections among troops in training units (Thomas, 1941; Anderson, Buchanan & MacPartland, 1944). The oiling of bed-linen in hospital laundries is now a practical possibility (Harwood, Powney & Edwards, 1944).

Enthusiasm for these new methods of cleansing the air must not blind us to the paramount value of adequate ventilation, particularly in winter, in the control of air-borne infection. To quote Florence Nightingale again "windows were made to be opened and doors to be shut," and patients in bed are remarkably tolerant of cold. Proper bed-spacing, i.e. 144 square feet [about 13 m.^{2}] per bed in infectious diseases hospitals and at least 100 square feet in children's and maternity hospitals, must also be maintained, for overcrowding increases the risk of cross-infection which, in turn, entails a longer stay in hospital and a slower turnover of patients, thus defeating the objective which prompted the increase in beds. Hospital wards should also be well-lit for, besides the bactericidal action of direct sunlight and daylight, Garrod (1944) has recently shown that daylight through ordinary glass is also bactericidal. Thus it is that, in the summer months with better ventilation, more daylight and less overcrowding, the incidence of respiratory cross-infection is always at its lowest. Our aim must be to utilize all the available methods of control in order to keep air-borne infection in hospitals at the same low level in both summer and winter.

Hospital Infection To-day

Advances in our knowledge of the mode of spread of infection, due in large part to laboratory research, have altered considerably the present-day conception of hospital infection. In particular, recognition of the human carrier as a reservoir of infection has helped to explain many anomalies and to dispel old notions about the dangers of miasma, sewer gases and the like. Hospital infection could to-day be defined *clinically* as manifest respiratory, gastro-intestinal, wound, skin, or mucous membrane infection, arising during the course of another disease, and, *bacteriologically*, as the acquisition by a patient of pathogenic organisms not present on admission.

If, with modern improvements in technique and accommodation, clinicians feel that cross-infection is no longer a serious problem, they may be reminded of such recent happenings as a secondary streptococcal infection-rate of over 70% in measles wards with one-fifth of the children developing otitis media (Wright, Cruickshank & Gunn, 1944); more than 60% of the clinical complications in scarlet fever due to cross-infection with an average stay in hospital of 52 days compared with 27 days for uncomplicated cases (de Waal, 1940); 13% of 551 children cross-infected with the haemolytic streptococcus during one year's admissions to a children's department (J. Wright, 1940); outbreaks of neo-natal diarrhoea with mortality-rates of over 50% (Ormiston, 1941; Sakula, 1943); and the all-too-frequent pyogenic infection of wounds in burns and oto-rhinological wards. Gastro-enteritis, dysentery, pemphigus neonatorum, diphtheria, are other hospital infections which are still too common.

Accompanying, and far transcending the obvious clinical infection, is the latent bacteriological infection serving as an undetected reservoir for the infection of new and susceptible cases. Thus, cross-infection may be likened to an iceberg with but one-ninth of its bulk above the surface for all to see. It is the submerged eight-ninths that spells danger, and it is the function of the bacteriologist to unmask this hidden infection in throat, gut or wound, for it is the unknown, not the known, carrier of pathogenic organisms who is most likely to be a danger to his fellows. This modern concept of hospital infection is developed in a recent *Medical Research Council* War Memorandum (1944), which discusses the sources and modes of spread and the general measures for prevention of hospital infection, gives specific advice about the control of contact and mediate, droplet and air-borne infection, and recommends procedure when a particular type of infection occurs in a ward. This practical pamphlet should be read by all who are interested or concerned in the problem of hospital infection, for only by intelligent and whole-hearted co-operation by everybody, from physician and surgeon to porter and domestic, can hospitals be made safer for those who require their healing aid. The complex problem of cross-infection is still unsolved, but to-day we understand more clearly what the contributory factors are. We still do not know the relative importance of better hospital design and equipment, well-trained nursing staff, new methods for controlling air-borne infection, chemotherapy and chemoprophylaxis, but if we apply the knowledge we already have we shall have gone a long way towards achieving Florence Nightingale's first requirement in a hospital that it should do the sick no harm.

REFERENCES

Allison, V. D. & Brown, W. A. (1937) *J. Hyg., Camb.* **37,** 153
Anderson, J. S., Happold, F. C., McLeod, J. W. & Thomson, J. G. (1931) *J. Path. Bact.* **34,** 667
Anderson, J. S., Cooper, K. E., Happold, F. C. & McLeod, J. W. (1933) *J. Path. Bact.* **36,** 169
[1] Anderson, P. H. R., Buchanan, J. A. & MacPartland, J. J. (1944) *Brit. med. J.* **1,** 616
Andrewes, C. H. (1940) *Lancet,* **2,** 770
Andrewes, C. H., & Glover, R. E. (1941) *Brit. J. exp. Path.* **22,** 91
Baker, A. H. & Twort, C. C. (1941) *J. Hyg., Camb.* **41,** 117
Bourdillon, R. B. & Lidwell, O. M. (1941) *Lancet,* **2,** 365
Brown, W. A. & Allison, V. D. (1937) *J. Hyg., Camb.* **37,** 1
[2] Browning, C. H. (1943) *Brit. med. J.* **1,** 341
Cameron, H. (1907) *On the evolution of wound treatment during the last forty years,* Glasgow
Crookshank, F. G. (1910) *Lancet,* **1,** 477
Crosbie, W. E. & Wright, H. D. (1941) *Lancet,* **1,** 656
Cruickshank, R. (1935) *J. Path. Bact.* **41,** 367
Cruickshank, R. (1940) *Lancet,* **2,** 54
Cruickshank, R. & Godber, G. E. (1939) *Lancet,* **1,** 741
Cruickshank, R. & Muir, C. (1940) *Lancet,* **1,** 1155
de Waal, H. L. (1940) *J. Hyg., Camb.* **40,** 172
Douglas, S. R., Hill, L. & Smith, W. (1928) *J. industr. Hyg.* **10,** 219
Dunkin, G. W. & Laidlaw, P. P. (1926) *J. comp. Path.* **39,** 213
[3] Edward, D. G. ff. & Lidwell, O. M. (1943) *J. Hyg., Camb.* **43,** 196
Fleming, A. & Porteous, A. B. (1919) *Lancet,* **2,** 49
Flügge, C. (1897) *Z. Hyg. InfektKr.* **25,** 179; (1899) *Z. Hyg. InfektKr.* **30,** 107
[4] Garrod, L. P. (1944) *Brit. med. J.* **1,** 245
Godlee, R. (1917) *Lord Lister,* London
Grancher, J. J. (1888) *Antisepsie médicale,* Paris; see also *Brit. med. J.* (1900) **2,** 1395
Greenwood, M. (1935) *Epidemics and crowd diseases,* London
Griffith, F. (1934) *J. Hyg., Camb.* **34,** 542
Hare, R. (1940) *Canad. publ. Hlth J.* **31,** 539
Harries, E. H. R. (1935) *Lancet,* **2,** 173 & 233
[5] Harwood, G. C., Powney, J. & Edwards, C. W. (1944) *Brit. med. J.* **1,** 615
Hornibrook, J. W., Nelson, K. R., Dyer, R. E., Topping, N. H. & Bengtson, I. A. (1940) *Publ. Hlth Rep. Wash.* **55,** 1936 & 1945
Hutinel, V. (1894) *Rev. mens. Mal. Enf.* **12,** 515; see also *Brit. med. J.* (1894) **2,** 993
Lancet (1940) *War primer on wound infection,* ed. by W. H. Ogilvie, London
McCoy, G. W. (1934) *J. infect. Dis.* **55,** 156
McIntosh, J. & Selbie, F. R. (1944) *Lancet,* **1,** 591
Masterman, A. T. (1938) *J. industr. Hyg.* **40,** 278
[6] Medical Research Council (1944) War Memorandum No. 11: *The control of cross-infection in hospitals,* London

Miles, A. A., Schwabacher, H., Cunliffe, A. C., Ross, J. P., Spooner, E. T. C., Pilcher, R. S. & Wright, J. (1940) *Brit. med. J.* **2**, 855 & 895
[7] Mitchell, G. A. G. & Buttle, G. A. H. (1942) *Lancet*, **2**, 416
Nightingale, F. (1859) *Notes on hospitals*, London
Nightingale, F. (1860) *Notes on nursing*, London
Nightingale, F. (1871) *On lying-in institutions*, London
Okell, C. C. & Elliott, S. D. (1936) *Lancet*, **2**, 836
Ormiston, G. (1941) *Lancet*, **2**, 588
Power, W. H. (1880–81) *Rep. med. Offr loc. Govt Bd.* **10**, Suppl. **2**, 302
Pugh, W. T. G. (1906) *Rep. metrop. Asylums Bd.*, p. 6
Robertson, O. H. (1942) in *Aerobiology*, ed. by F. R. Moulton, Washington
Rundle, C. & Burton, A. H. G. (1912) *Lancet*, **1**, 720
Sakula, J. (1943) *Lancet*, **2**, 758
Smith, J. L., Drennan, A. M., Rettie, T. & Campbell, W. (1915) *Brit. med. J.* **2**, 129
Smith, J. L., Ritchie, J. & Rettie, T. (1917) *Brit. med. J.* **2**, 386
Stokes, A. & Tytler, W. H. (1918) *Brit. J. Surg.* **6**, 92
Thomas, J. C. (1941) *Lancet*, **2**, 123
Thomson, F. H. (1916) *Lancet*, **1**, 341
Twort, C. C., Baker, A. H., Finn, S. R. & Powell, E. O. (1940) *J. Hyg., Camb.* **40**, 253
Twort, C. C. & Baker, A. H. (1942) *J. Hyg., Camb.* **42**, 266
Tyndall, J. (1881) *Essays on the floating-matter of the air in relation to putrefaction and infection*, London
[8] van den Ende, M., Harries, E. H. R., Stuart-Harris, C. H., Steigman, A. J. & Cruickshank, R. (1943) *Lancet*, **1**, 328
van den Ende, M., Lush, D. & Edward, D. G. ff. (1940) *Lancet*, **2**, 133
van den Ende, M. & Spooner, E. T. C. (1941a) *Lancet*, **1**, 751
van den Ende, M. & Thomas, J. C. (1941b) *Lancet*, **2**, 755
Washbourn, J. W. & Eyre, J. W. H. (1902) *Lancet*, **2**, 1439
Wells, W. F. & Wells, M. W. (1936) *J. Amer. med. Ass.* **107**, 1698 & 1805
White, E. (1936) *Lancet*, **1**, 941
Wright, H. D., Shone, H. R. & Tucker, J. R. (1941) *J. Path. Bact.* **52**, 111
Wright, J. (1940) *J. Hyg., Camb.* **40**, 649
[9] Wright, J., Cruickshank, R. & Gunn, W. (1944) *Brit. med. J.* **1**, 611

[1] [see *BMB* **580**] [2] [see *BMB* **56**] [3] [see *BMB* **557**] [4] [see *BMB* **577**] [5] [see *BMB* **579**]
[6] [see *BMB* **387/39**] [7] [see *BMB* **57**] [8] [see *BMB* **68**] [9] [see *BMB* **578**]

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OBSERVATIONS ON THE CONTROL OF HOSPITAL INFECTION

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The problems of hospital infection are like the problems of transmissible disease in general; they cannot be appreciated without a knowledge of the reservoirs of infection, of the channels whereby the infective agents reach the susceptible person, and of the mode of infection. The hospital problem is peculiarly acute in that a large variety of infections may be gathered together in one institution, and that infected and susceptible persons are, geographically speaking, crowded together in a small area. Moreover, the infected, because they are ill enough to require hospital treatment, are likely to be heavily infected, and the susceptible, being treated for other, perhaps debilitating, diseases, are likely to be more susceptible than healthy persons in the population at large. These epidemiological disadvantages of hospitals are of course counter-balanced by the advantages of supervision of the hospital community by skilled medical staff. But though in the past century the reforms of hospital administration and technique have eliminated the grosser horrors of hospital infection, it is not fully recognized to-day that, even in modern hospitals, infection of this sort frequently occurs, and that in many hospitals the preventive measures in force would prove inadequate to prevent the spread of an infection among the patients or staff, if an infective agent were introduced among them.

Added Infection: Magnitude of the Problem

The infections with which we are concerned are qualified by the word "hospital," which is intended to indicate, not that they are necessarily acquired in a given type of institution, but that they are acquired by a patient as a result of submitting to *any* form of medical or surgical treatment. The word "hospital" is too restricted; the important feature of the infection is that it is *added* to a patient already suffering from the disease for which he seeks treatment. In this article, "added infection" is used synonymously with "hospital infection," and is preferred, because it keeps clearly before the reader the idea of an unnecessary and to a large extent an avoidable burden imposed on the patient.

It is often assumed that the risk of added infection is adequately met if the great majority of patients never suffer from a severe disease traceable to an infection picked up in the hospital. This is erroneous for a number of reasons. In the first place, the sources of infection may be absent or scanty for long periods, and the precautions, though poor, are consequently never tested against a heavy load of infection. For instance, in surgical wards, the incidence of infection in "clean" operation wounds rises in war-time, when patients with such wounds are nursed alongside patients with heavily infected war-wounds (McKissock, Wright & Miles, 1941). In the second place, an infection acquired in hospital may make little obvious change in already sick patients, though it may prolong the period of illness, or eventually kill the patient. An obvious illustration is again found in wounds; the addition of *Strept. pyogenes*, to a wound already septic as the result of infection with *Staph. aureus*, may not be at first clinically apparent, but the subsequent history of the wound is that of a severe streptococcal infection (Miles, Schwabacher, Cunliffe, Ross, Spooner, Pilcher & Wright, 1940; Spooner, 1941). Again, children convalescent from infection by one type of diphtheria bacillus, may be re-infected by diphtheria bacilli of another type (Glass & Wright, 1938; Wright, Shone & Tucker, 1941). In the third place, the patient's response to added infection may not take the form of a gross, acute disease. He may become a healthy carrier of the infecting organism, or he may suffer from an infection which is clinically silent. In a children's ward observed for one year, Wright (1940) found that of all the children who acquired a *Strept. pyogenes* in the throat, only 25% showed any signs of infection. The healthy carrier, by definition, does not suffer from the infection, but he becomes a reservoir of the organisms and a menace to other patients who may be highly susceptible to the infection. To the susceptible patient, the carrier and the silently-infected patient are in fact a greater menace than the manifestly-infected patient, for they display no danger signs whereby their condition may be recognized, and the risks of transfer guarded against.

Silent infections and the healthy carrier-state are indistinguishable by a single clinical or bacteriological examination but, in some cases at any rate, it is clear that some of those who carry pathogens without suffering from manifest disease, do in fact suffer disabilities from the presence of the organism in the body. For instance, in one survey (Gissane, Miles & Williams, 1944) the average period of treatment for minor lacerations of the hand treated by excision and suture was

Clinically and bacteriologically clean wounds .	15·6 days
Clinically clean, but "infected" wounds . .	18·2 days
Septic wounds	25·4 days

Here the average healing time of the silently infected wounds is 20% higher than that for clean wounds, a sufficiently striking figure for small wounds; the healing time is increased 60% in the septic wounds. For larger wounds, there are few precise records of the effect of added infection, though there is general agreement that infection *per se* delays healing. In one series of air-raid wounds of the head, the average healing time of those with added streptococcal and staphylococcal infection was over 100 days; those without added infection healed in an average of 35 days (McKissock *et al.*, 1941).

It follows from these preliminary remarks (*a*) that preventive measures must cover more than obvious existing risks; to meet greater possible risks they must be made as infection-proof as is practicable; (*b*) that preventive measures must

aim at reducing the spread of mild as well as severe infection, and at preventing the increase of the carrier-state and of silent infections; and finally (*c*) that the criteria of an added infection are in the last analysis bacteriological, not clinical.

The crippling or fatal added infection, however rare its occurrence, is an index of gaps in the hospital's defence against infection, gaps through which, it would be salutary to assume, mild and silent infections are all the time creeping. There is no good reason why a patient or a member of the medical staff should suffer, even mildly, from an avoidable infection acquired from other patients or members of the hospital staff. To allow him to do so is both inhumane and uneconomical. In times of war, when the demand on manpower and medical services becomes acute, avoidable infection is even less defensible.

Sources of Added Infection

The three main types of added infections are, in order of their importance and prevalence: respiratory; gastro-intestinal; and infections of skin, external mucous membranes and wounds. With the exception of wounds and burns—infections which attain a peculiar predominance in war-time—these infections bear most heavily on the child population (*Medical Research Council*, 1944). From the hospital point of view, the reservoirs of all these infections are excreta and discharges (including droplets from the upper respiratory tract) from manifest cases of the various diseases, or from healthy carriers. From the upper respiratory tract come bacteria, such as *Strept. pyogenes*, *Staph. aureus*, pneumococci and meningococci, the bacilli of diphtheria, whooping-cough and tuberculosis; and viruses, or presumed viruses, like the agents of measles, chicken-pox, rubella, mumps, infectious hepatitis. From faeces, vomit and urine may come *Salmonella* and dysentery bacilli, protozoa like *Giardia*, and perhaps the causal agent of the so-called non-specific enteritis. And from infected skin, conjunctivae, mouths, external genital tracts, and from infected wounds may come discharges bearing bacteria of the numerous pathogenic species, both aerobic and anaerobic, known to infect these places.

Preventive Measures: Some Important Considerations

It is obviously impossible to eliminate these reservoirs of infection from among the patients whose infection may have brought them to hospital. Elimination of carriers from the hospital staff is both possible and desirable, but in some cases it is impracticable. It may be impracticable if the carrier-state is so common that to dismiss the carriers would necessitate closing the hospital. The nasal carrier-rate of potentially pathogenic *Staph. aureus* in a nursing staff may be as high as 80% (Miles, Williams & Clayton-Cooper, 1944). No maternity home, for example, faced with an outbreak of staphylococcal "pemphigus" among the infants, could afford to send 80% of its nurses off duty. The same consideration might apply to carriers of the meningococcus. In other cases, as with Salmonella bacilli, the probability of added infection is too great, and the consequences of infection are too serious, to allow a carrier to continue in hospital service.

It is outside the purpose of this article to discuss the cure of intestinal or respiratory carriers. In any event, evidence of the complete cure of a carrier state, especially in the upper respiratory tract, is hard to obtain, as absence of the organism in question from the samples examined bacteriologically may indicate only a temporary absence of the organism from the superficies of the mucous membrane. And it is known, for example, that *Strept. pyogenes* (Straker, Hill & Lovell, 1939) and pneumococci (Smillie, Calderone & Onslow, 1943) may reappear in the throat after a prolonged naturally-occurring absence. Nevertheless, measures which apparently cure or diminish a carrier-state have their use, as at least they reduce the load of bacteria which the carrier disseminates through his surroundings. Of simpler measures which appear to achieve this end in the upper respiratory tract, we may cite the successful use of penicillin (Delafield, Straker & Topley, 1941) and sulphathiazole snuffs (Delafield & Straker, 1941; Thomas, 1941; Goldman & Patterson, 1942) for the treatment of nasal carriers of *Staph. aureus*, *Strept. pyogenes* and *C. diphtheriae*, the treatment of throat carriers of *C. diphtheriae* by aerosols of sulphapyridine (Legros, 1943) and of throat carriers of *Strept. pyogenes* by tonsillectomy and sulphanilamide treatment (Chesterman & Scandrett, 1940).

Since only a limited attack can be made on the reservoirs of infection, preventive measures are usually devised closing the channels of infection. It is customary to distinguish spread of infection by (*a*) contact, which may be direct (immediate) or indirect (mediate)—such as transmission by hands, instruments, utensils, clothes, food, flies, etc.; (*b*) by droplets; and (*c*) by the air.

It is certain that a number of infective agents, among them bacteria and viruses, can remain infective for long periods as dry or semi-dry particles, small enough to be carried considerable distances by relatively feeble currents of air. These particles may be formed by the disintegration of dried infective discharges and excretion. Explosive respiratory discharges, such as accompany sneezing, coughing and talking, are particularly prolific in the production of the particles. Fluid droplets of various sizes are discharged in these explosions. Wells (1934) distinguished the relatively large liquid droplet from the small particles, which in the course of a few seconds dry up and form a readily air-borne "droplet nucleus". It is the infected droplet nuclei that infest the air. The importance of the distinction has recently been emphasized by Hare (1940), who showed by bacteriological methods that the larger droplet depends upon the explosive impulse for its dissemination, and has a horizontal range of about 1 metre only. The maximum horizontal range, determined by flash-photography of droplets during expulsion, is about 2 metres (Bourdillon & Lidwell, 1942). The droplet is not, in fact, truly airborne (Mackie, 1942) and may logically be considered as an example of mediate contact infection. There are, however, many other sources of airborne infective particles besides the droplet-nucleus. The large droplet that falls to the ground or on to bedclothes, and infective discharges that contaminate dressing, clothes and bedding, disintegrate and eventually add their quota of infective dust to the air. The prevalence and dangers of dust-borne *Strept. pyogenes* (Cruickshank, 1935; Allison & Brown, 1937; White, 1937; Cruickshank & Godber, 1939; Cruickshank & Muir, 1940; Kelsey, 1941; Willits & Hare, 1941; Edward, 1944) and *C. diphtheriae* (Crosbie & Wright, 1941) are well established.

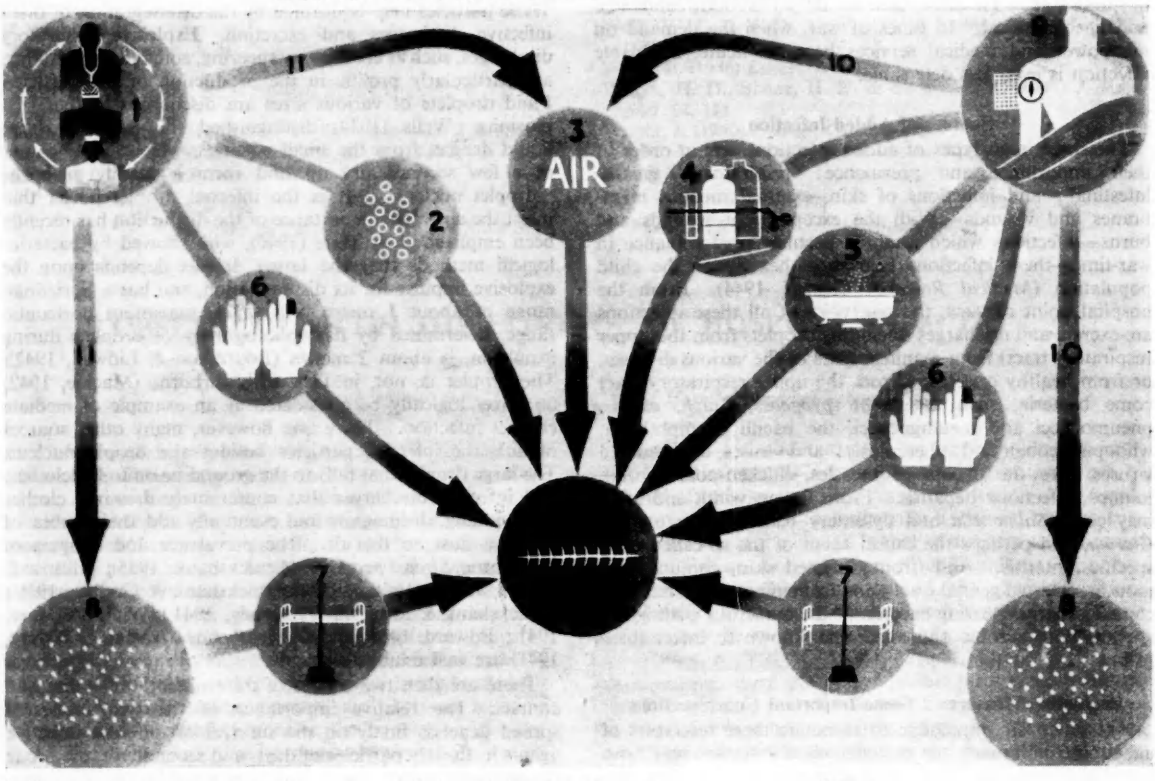
There are then two modes of transmission, air-borne and contact. The relative importance of the two modes of spread depends firstly on the survival of different infective agents in the dry particles of dust, and secondly on the mode of infection in each disease. It appears (Mackie, 1942) that staphylococci, haemolytic streptococci, pneumococci, the diphtheria bacillus, bacterial spores, and tubercle bacilli survive for relatively long periods in the dry state. *Haemophilus*, the plague bacillus, Salmonella and dysentery bacilli, and the cholera vibrio die rapidly. Among the viruses, those of variola, vaccinia, psittacosis and poliomyelitis appear to survive well; the influenza virus (Edward, 1941) is moderately resistant. With regard to the mode of infection, air-borne infections are paramount in respiratory disease, as even a dilute infestation of the air will tend with continuous breathing to concentrate in the upper respiratory tract of the patients at risk, and the opportunity for the aerial dissemination of infective agents from those already infected is obviously great. Air-borne infections probably occur to some extent in wounds (Miles *et al.*, 1940). In the recorded outbreaks of added infection of wounds, however, the observed opportunities for contact infection have been so great that the rôle of air-borne bacteria cannot be judged with any accuracy. It may nevertheless be assumed to have been small, because infective particles fall slowly from the air on to the exposed wound; though big wounds and extensive burns, the large area of which entails long periods of exposure during dressing, must be considered as relatively susceptible to air-borne infection.

There appears to be little opportunity for the rapid drying of infective intestinal discharges, and their subsequent disintegration into air-borne particles. Feeding utensils and food are of prime importance in the spread of intestinal diseases. Other vehicles are important as they are related to the ingestion of infective material, to excretion on the part of the patient, and to the handling and disposal of excreta by the hospital staff.

The possible vehicles of skin and wound infection are, like those of intestinal infections, too numerous for detailed mention. Even a perfunctory survey of an operating-theatre, out-patient or ward routine will reveal an abundance of opportunity for the transmission of infective material,

if it is assumed that infective material can be introduced almost at any point in the routine. As we have seen, the silently infected patient is a danger because his potentialities are unrecognized; in the same way, the silently contaminated hand, utensil or instrument is equally dangerous. To take an example, in a ward containing one or two wounds infected with *Strept. pyogenes*, the coccus may be grown, not only from the wound and the obviously soiled inner dressings of sampler (Bourdillon, Lidwell & Thomas, 1941) as well as other devices for efficient sampling of the bacterial content of the air, have permitted tests of various methods of diminishing or killing air contaminants. Some of these methods, like the installation of air-conditioning plants capable of removing bacterial particles, are prohibitively expensive for most hospitals, and in war-time the necessary apparatus may be unobtainable. In this category, also, comes the use

HOW A CLEAN WOUND MAY BECOME INFECTED



1. Upper-respiratory-tract infection cycle: Doctor-patient-nurse.
2. Droplets.
3. Air.
4. Instruments, "sterile" lotions, etc.
5. Toilet utensils, baths, etc.
6. Hands: septic, and normal but contaminated.
7. Sweeping and bed-making.
8. Dust.
9. Infected wounds, dressings, bed-clothes, etc.
10. Small particles.
11. Droplet nuclei.

the infected patient, but also from the apparently clean outer dressing, the bandage, the patient's skin for distances up to 30 cm. from the wound, his pyjamas, pillow-cases, sheets, blankets, bed-rail; from the dust under his bed, the curtains of his screens placed round the bed, from the grease-line of wash-bowls and baths (often in communal use in the ward) that he uses. It will be obvious that a nurse who has nothing to do with the actual dressing of that wound may carry the streptococcus to other patients merely by touching the outer bandage, adjusting the bedclothes or moving the bed.

Available Modes of Closing Channels of Infection

Air-borne infection: Air-borne infection must depend for its success on a relatively high concentration of the infective particles in the environment of the susceptible person, and for this reason it is likely that a great many of its dangers can be guarded against by dilution of the infective agent. It is well recognized that the bacteria of air and dust are sterilized in sunlight, but less well recognised that diffuse daylight is bactericidal (Solowey, Solotorovsky & Buchbinder, 1942) even after passage through media which, like ordinary window glass, are impervious to ultra-violet rays (Garrod, 1944). Ventilation, intelligently applied, has been and will remain a simple bulwark against air-borne infection. There are, however, many situations for which the remedy of ventilation is inadequate or inapplicable, and in recent years many attempts have been made, either to sterilize contaminated air, or to shield the patient from it. Indeed, spectacular advances in the control of added infection have been made particularly in the realm of air-borne infection. The Wells air centrifuge (Wells, 1933) and the Bourdillon slit-of batteries of ultra-violet lamps for air sterilization, though there is good evidence that ultra-violet irradiation is lethal to aerial suspensions, for example, of viruses (Edward, Lush & Bourdillon, 1943) and that the incidence of various respiratory infections of children in wards and schoolrooms —measles (Wells, Wells & Wilder, 1942), varicella (Green, Barenberg & Greenberg, 1941) and general upper respiratory infections (Barenberg, Greene & Greenspan, 1940; del Mundo & McKhann, 1941; Robertson, Doyle & Tisdall, 1943)—is markedly reduced by irradiation of the air in wards and schoolrooms. On the other hand, Sommer and Stokes (1942) report no detectable effect of irradiation on the incidence of pneumococcal infection in children's wards, though both irradiation and glycol vapours reduced the infection rate in mice that had been exposed in wards contaminated with droplet-nuclei containing influenza virus or streptococci (Henle, Sommer & Stokes, 1942). Glycol vapours in concentrations tolerable by the human being are lethal to artificial suspensions of bacteria (Puck, Robertson & Lemon, 1943) and influenza virus (Robertson, Loosli, Puck, Bigg & Miller, 1941), and their use in a children's ward (Harris & Stokes, 1943) was accompanied by a marked reduction in the incidence of tracheobronchitis and the common cold, as compared with a control ward. Hypochlorite sprays are effective against suspended viruses and bacteria (Baker, Finn & Twort, 1940; Challinor, 1943; Edward & Lidwell, 1943) and have been used with apparent success in suppressing epidemics of respiratory infection in military camps (Middleton & Gilliland, 1941). Pulvertaft (1944) emphasizes the simplicity of their application—by atomization of hypochlorite solutions in household insecticide-spraying pumps. Lovelock, Lidwell & Raymond (1944) have recently demonstrated

that lactic acid sprays have a marked bactericidal effect on artificially- and naturally-infected air. The different lethal agents vary in the range of conditions over which they are effective, but to some extent all of them are less effective in dry atmospheres, and in air containing high proportions of inert dust-particles.

There are even simpler methods of reducing the contamination of the air. Bacteria-containing dust sediments with comparative rapidity. For instance, though the bacterial content of ward air rises considerably at bed-making and sweeping, it usually drops to a low level in half to one hour after activity in the ward is reduced to a minimum. Advantage may be taken of this sedimentation-rate by the application of oils to the floor and other smooth surfaces of the ward, and to bed-linen, blankets and curtains. The oil not only prevents the dissemination of bacterial dust during ward activities like sweeping and bed-making, but also (Thomas, 1941) holds newly-formed bacterial dust as it settles after its discharge from the carrier or the infective patient (van den Ende, Lush & Edward, 1940; van den Ende, Edward & Lush, 1941; van den Ende & Spooner, 1941; van den Ende & Thomas, 1941). Paraffin or commercial white oil is used. The method is suitable for wooden and linoleum floors, and both cotton and woollen bed-clothing and garments can, by a simple and cheap laundry technique (Harwood, Powney & Edwards, 1944), be impregnated with bacteria-holding concentrations of oil. Material so treated is highly effective in holding bacterial particles. By these means, Wright, Cruickshank & Gunn (1944) have recently produced a marked reduction in the incidence of streptococcal added infection in one measles ward, as compared with that in a similar " unoiled " control ward. Anderson, Buchanan & MacPartland (1944) for 4 winter months observed respiratory infections in two military units which differed only in that the floors of all the huts in one of them were oiled at monthly intervals. The average weekly rate of acute respiratory infection per 1,000 men was 7 in the " oiled " unit, and 38 in the control.

Contact-infection: There are no spectacularly successful, or simply applied techniques for the reduction of contact-infections. The channels of contact-infections are many and various, and each may require a separate mode of closing. It is probable that great improvements would follow the abolition of large open wards and the division of hospitals into a number of single-bedded units and small wards (*Medical Research Council*, 1944). But no subdivision of this kind will be effective unless all possible contact-transmissions are prevented. To take an obvious example, the most elaborate design of ward will fail to check the spread of contact-infections in a children's ward if the duties of one nurse include routine attendance on the patient's excretions, and preparation of his food. The principles which govern the design of precautionary methods are usually simple; the chief difficulty in applying them is failure to recognize where the risks of infection are greatest, and it is here, at any rate with infective agents that are readily cultivable, that a bacteriological survey is chiefly valuable. The main reason for the failure of precautions is failure to work intelligently from the principles. For instance, the ritual observance of a rule that every nurse should wash her hands after a visit to the water-closet will have little effect on added infection of the nursing staff by Sonne's dysentery bacilli if the wash-basin is outside the water-closet, for the door handle in each case is thoroughly contaminated before the hands are washed.

As an illustration of the complexity of the problem, we may consider the elimination of hospital infections of clean wounds in a surgical ward. Our primary aim is to protect the wound from potentially pathogenic bacteria; we cannot hope to eliminate all bacteria from the ward environment at the time of dressing.

With few exceptions (e.g. the brain), freshly cut tissues of the body appear to be bactericidal enough to kill small numbers of saprophytic organisms, without the mediation of detectable inflammatory reaction. The uneventful healing of large numbers of operation-wounds, made in theatres that are certainly not bacteria-free, is sufficient evidence of this point, though the possibility of harmful " silent " infection cannot be excluded in operation-wounds that heal by " first intention." Bearing in mind the " silent " infections, Gudin (1942) pleads strongly for the elimination of *all* possible sources of infection, and insists that a true sterile healing of wounds cannot be guaranteed unless the operation is performed in a special theatre whose walls, furniture and instruments are sterile, by persons completely covered to contain their personal bacteria, upon a patient whose skin is completely sterile. Gudin's " integral asepsis " is devised for theatre work; and though his analysis of the dangers of theatre contamination may be read with profit by those designing aseptic methods for the ward, the method is not strictly applicable to wards, where the problem is one of local bacteriological control of a small area of operation.

We have for the most part to guard against *Staph. aureus* and *Strept. pyogenes* which, together with the faecal coliform bacilli, are the chief cause of hospital infection of wounds (Miles *et al.*, 1940; Williams, Clayton-Cooper, Howat & Miles, 1945). The figure [see page 278] illustrates the situation of a clean wound exposed for dressing in such a ward. There are two main reservoirs of these organisms, namely, already infected wounds, and the upper respiratory tract of patients and personnel. The carriage of *Strept. pyogenes* in the throats of 5-15 % of normal persons is well recognized. It is less well recognized that *Staph. aureus* is carried, usually in profusion, in the nasal cavity of about 50 % of normal adults, and that the carriage is more or less persistent (Miles, Williams & Clayton-Cooper, 1944). The dangers of infected droplets, droplet-nuclei, from these sources have already been discussed. The other reservoir, the infected wound, is a profuse source of the two cocci. It cannot be too strongly emphasized that not only are the wound exudate, and the inner dressings of such wounds, full of organisms, but also that the skin for a large area around the wound, the skin of the patient's hands, and all the bed-clothes, may be holding the infected dust. Some of the places where *Strept. pyogenes* may lie in such a ward have been listed above. *Staph. aureus* will also be found in these places. And we may add the personal bandage-scissors of the nurses; the aprons, coats and gowns of the staff, the hands of staff; instruments, lotions, and gauze or lint contaminated in bulk when samples are taken with soiled instruments or hands during the course of a dressing. The two cocci may spread by means of instruments and hands of medical staff. They may spread via wash-bowls and baths, where they collect in the grease-line, and defy all but the most rigorous antiseptic treatment.

Bacteriologically Acceptable Dressing-Techniques

There are two alternatives for the safe dressing of wounds. Either the wound may be dressed in a theatre or side-room, the whole of which is under full control, or the dressing may be done in a ward, where an attempt is made to create local conditions of asepsis round the exposed wound. The dressing theatre has many advantages, and it may prove to be a necessity in the treatment of burns, which are peculiarly susceptible to added infection. Nevertheless, it is often convenient to dress wounds in a general ward, and it should be possible to do so in comparative safety from infection.

Some of the precautions necessary to achieve local control of infection in the surgical ward are obvious. Droplet-infection is prevented by rigorous masking, using masks with at least one layer of an impermeable material like cellophane. Soiled dressing-materials—plasters, bandages, etc., must be removed and disposed of with the minimum of disturbance into deep, capacious discard-bins. Plasters, which may be impregnated with dried bacteria, should be damped before removal. The wound must be exposed as little as possible, and dressed by a no-touch technique[1] (as distinct from the " dripping-finger " method of manipulating the wound with scrubbed wet hands). The essentials of the no-touch technique are as follows. Dry sterile forceps must be used for all manipulations of the wound. The hands of the dresser need not be surgically sterile, but must be clean and dry. All material for the dressing of the wound is passed in forceps to the dresser by a person whose sole duty is to look after a trolley containing all clean and sterile material. A third person should be available to help by adjusting clothes, bandaging, and sterilizing instruments, etc., for use. This third person, like the dresser, must wash carefully between each dressing, or he may become a dangerous vehicle of infection, as from each infected patient he may be liberally contaminated by touching bed-clothes, pyjamas, healthy skin, and all the outer dressings. Persons

[1] [The no-touch technique of dressing wounds has been described in detail by the *Medical Research Council* (1941).]

with infections of the hand must not be allowed to dress the wound; and all persons who handle the wound, in any capacity, must be thoroughly familiar with the elements of prophylaxis against contact infection (*Medical Research Council*, 1941).

The more thorough methods of eliminating dust have been discussed above. Apart from these, much can be done by damp-dusting and -sweeping, and the cessation of all dust-raising activities in the ward half-an-hour before, and throughout, the period when wounds are dressed. It is most important that the precautions should be observed by *all* hospital staff concerned. Often, when the most rigorous precautions are taken to prevent the dresser of the wound from spreading infection, great latitude in this respect will be allowed to the visiting surgeon, the porters, to *x*-ray attendants, masseurs and ward-maids.

Simple revisions on these lines have proved successful in reducing added infection of wounds. McKissock *et al.* (1941) reduced a gross streptococcal infection-rate of 31% to 2% in an air-raid casualty ward. In a septic ward treating industrial accidents, where the reservoirs of infection were numerous, the incidence, per wound-weeks at risk, of *Strept. pyogenes* infection was 14%, and of *Staph. aureus* infections, 100%. After a revision of ward technique, these figures fell to 18% and less than 1%, respectively (Williams *et al.*, 1945). Ascroft (1944) has also made striking reductions in "clean" wound infection by simple revision of certain nursing procedures. Revision need not mean elaboration and a consequent increase in work for the hospital staff, for many existing precautions are often redundant, and their elimination allows ample time for taking more rewarding precautions. Revised dressing methods, in fact, once they are learned, are quicker in performance than some of the older methods (Gissane *et al.*, 1944).

Direct Protection of the Patient against Added Infection

We have so far discussed elimination of the reservoirs of infection, and stoppage of the channels of infection. There remains a third method, namely, the enhancement of the patient's powers of resistance either by specific immunization against prevalent hospital infections, such as diphtheria and pertussis, or by the administration of prophylactic doses of chemotherapeutic agents. Both are beyond the scope of this article, but mention of the second raises a point of some importance in wound infection. It is believed by some that the success of the sulphonamides and penicillin against the Gram-positive organisms to some extent excuses a perfunctory method of aseptic handling of wounds. The view is quite unjustified, for some strains of the pyogenic organisms are, or may become, drug-fast; moreover, as the extensive survey of Meleney and his colleagues suggests (Meleney, 1943), the prophylactic use of sulphonamides may have little effect on the incidence of added or late-developing infections; and finally, the organisms that are relatively insensitive to these agents—the coliform bacteria, *Ps. pyocyanea*, *Proteus vulgaris*, *H. para-influenzae*, etc.—are by no means negligible as causes of severe infection, and of severe residual disabilities in the ultimately healed wound.

Conclusion

In conclusion, it should be emphasized that the difficulties of preventing added infections come more from the psychological obstacles to easy reform, than from the technical problems of prophylaxis. It is essential that the staff of hospitals should be educated to recognize the reservoirs of infection, their magnitude and dangers, and to meet the dangers by methods that are flexible enough to change when new risks of added infection are discovered. Rules of procedure must be intelligible as applied principles; they must not be mere lists of commands; if they are, they will fail because they will be obeyed blindly when changing circumstances dictate their modification.

A great deal can be achieved without elaborate apparatus, and without specially-trained staff. Elaborate measures like ultra-violet irradiation, or the use of dressing theatres, may be necessary. Such measures are often credited with a success to which they are not fully entitled; for consciousness, on the part of the staff, of the infection risks that the elaborate measures have been designed to meet, may lead to a general improvement in the performance of the simpler precautionary duties.

In the future, precautions will be more easily taken if hospitals are designed for preventive as well as for administrative convenience. But no elaboration of design will be effective unless the education of the doctor and the nurse inculcates not rules for prevention, but the general principles from which the *ad hoc* methods of attack can be readily devised.

References

Allison, V. D. & Brown, W. A. (1937) *J. Hyg., Camb.* **37**, 153
[1] Anderson, P. H. R., Buchanan, J. A. & MacPartland, J. J. (1944) *Brit. med. J.* **1**, 616
Ascroft, P. B. (1944) *Lancet*, **1**, 594
Baker, A. H., Finn, S. R. & Twort, C. C. (1940) *J. Hyg., Camb.* **40**, 560
Barenberg, L. H., Greene, D. & Greenspan, L. (1940) *Amer. J. Dis. Child.* **59**, 1219
Bourdillon, R. B. & Lidwell, O. M. (1941) *Lancet*, **2**, 365
Bourdillon, R. B., Lidwell, O. M. & Thomas, J. C. (1941) *J. Hyg., Camb.* **41**, 197
[2] Challinor, S. W. (1943) *J. Hyg., Camb.* **43**, 16
Chesterman, J. N. & Scandrett, S. (1940) *Med. J. Aust.* **2**, 695
Crosbie, W. E. & Wright, H. D. (1941) *Lancet*, **1**, 656
Cruickshank, R. (1935) *J. Path. Bact.* **41**, 367
Cruickshank, R. & Godber, G. E. (1939) *Lancet*, **1**, 741
Cruickshank, R. & Muir, C. (1940) *Lancet*, **1**, 1155
Delafield, M. E. & Straker, E. (1941) *Brit. med. J.* **2**, 221
Delafield, M. E., Straker, E. & Topley, W. W. C. (1941) *Brit. med. J.* **1**, 145
del Mundo, F. & McKhann, C. F. (1941) *Amer. J. Dis. Child.* **61**, 213
Edward, D. G. ff. (1941) *Lancet*, **2**, 664
[3] Edward, D. G. ff. (1944), *J. Hyg., Camb.* **43**, 256
[4] Edward, D. G. ff. & Lidwell, O. M. (1943) *J. Hyg., Camb.* **43**, 196
[5] Edward, D. G. ff., Lush, D. & Bourdillon, R. B. (1943) *J. Hyg., Camb.* **43**, 11
[6] Garrod, L. P. (1944) *Brit. med. J.* **1**, 245
[7] Gissane, W., Miles, A. A. & Williams, R. E. O. (1944) *Brit. J. indust. Med.* **1**, 90
Glass, V. & Wright, H. D. (1938) *J. Hyg., Camb.* **38**, 248
Goldman, J. & Patterson, W. H. (1942) *Brit. med. J.* **1**, 641.
Green, D., Barenberg, L. H. & Greenberg, B. (1941) *Amer. J. Dis. Child.* **61**, 273
Gudin, M. (1942) *Ann. Surg.* **115**, 452
Hare, R. (1940) *Canad. publ. Hlth J.* **31**, 539
Harris, T. N. & Stokes, J. (1943) *Amer. J. med. Sci.* **206**, 631
[8] Harwood, F. C., Powney, J. & Edwards, C. W. (1944) *Brit. med. J.* **1**, 615
Henle, W., Sommer, H. E. & Stokes, J. (1942) *J. Pediat.* **21**, 577
Kelsey, H. (1941) *Med. J. Aust.* **1**, 728
Legros, J. (1943) *Presse méd.* **51**, 332
Lovelock, J. E., Lidwell, O. M. & Raymond, W. F. (1944) *Nature, Lond.* **153**, 20
Mackie, T. J. (1942) *Edinb. med. J.* **49**, 607
McKissock, W., Wright, J. & Miles, A. A. (1941) *Brit. med. J.* **2**, 375
Medical Research Council (1941) War Memo. No. 6: *The prevention of "hospital infection" of wounds*, London
[9] Medical Research Council (1944) War Memo. No. 11: *The control of cross infection in hospitals*, London
Meleney, F. (1943) *Ann. Surg.*, **118**, 171
Middleton, D. S. & Gilliland, I. C. (1941) *Lancet*, **2**, 598
Miles, A. A., Schwabacher, H., Cunliffe, A. C., Ross, J. P., Spooner, E. T. C., Pilcher, R. S. & Wright, J. (1940) *Brit. med. J.* **2**, 855 & 895
Miles, A. A., Williams, R. E. O. & Clayton-Cooper, B. (1944) *J. Path. Bact.* **56** [in press]
Puck, T. T., Robertson, O. H. & Lemon, H. M. (1943) *J. exper. Med.* **78**, 387
[10] Pulvertaft, R. J. V. (1944) *J. Hyg., Camb.* **43**, 352
Robertson, E. C., Doyle, M. E. & Tisdall, F. F. (1943) *J. Amer. med. Ass.* **121**, 908.
Robertson, O. H., Loosli, C. G., Puck, T. T., Bigg. E. & Miller, B. F. (1941) *Science*, **94**, 612
Smillie, W. G., Calderone, F. A. & Onslow, J. M. (1943) *Amer. J. Hyg.* **37**, 156
Smith, C. R. (1942) *Amer. Rev. Tuberc.* **45**, 334
Solowey, M., Solotorovsky, M. & Buchbinder, L. (1942) *J. Bact.* **43**, 545
Sommer, H. E. & Stokes, J. (1942) *J. Pediat.* **21**, 569
Spooner, E. T. C. (1941) *J. Hyg., Camb.* **41**, 320
Straker, E. A., Hill, A. B. & Lovell, R. (1939) *Rep. publ. Hlth med. Subj., Lond.*, No. 90
Thomas, A. M. (1941) *Brit. med. J.* **2**, 687
Thomas, J. C. (1941) *Lancet*, **2**, 123
van den Ende, M., Edward, D. G. ff. & Lush, D. (1941) *Lancet*, **1**, 716

van den Ende, M., Lush, D. & Edward, D. G. ff. (1940) *Lancet*, **2**, 133
van den Ende, M. & Spooner, E. T. C. (1941) *Lancet*, **1**, 751
van den Ende, M. & Thomas, J. C. (1941) *Lancet*, **2**, 755
Wells, W. F. (1933) *Amer. J. publ. Hlth*, **23**, 58
Wells, W. F. (1934) *Amer. J. Hyg.* **20**, 611
Wells, W. F., Wells, M. M. & Wilder, T. S. (1942) *Amer. J. Hyg.* **35**, 97
White, E. (1937) *Lancet*, **1**, 941
Williams, R. E. O., Clayton-Cooper, B., Howat, T. W. & Miles, A. A. (1945) *Brit. J. Surg.* [in press]
Willits, R. E. & Hare, R. (1941) *Canad. med. Ass. J.* **45**, 479
Wright, H. D., Shone, H. R. & Tucker, J. R. (1941) *J. Path. Bact.* **52**, 111
Wright, J. (1940) *J. Hyg., Camb.* **40**, 647
[11] Wright, J., Cruickshank, R. & Gunn, W. (1944) *Brit. med. J.* **1**, 611

[1] [*see BMB* 580] [2] [*see BMB* 30] [3] [*see BMB* 576] [4] [*see BMB* 557] [5] [*see BMB* 67] [6] [*see BMB* 577] [7] [*see BMB* 583] [8] [*see BMB* 579] [9] [*see BMB* 387/39] [10] [*see BMB* 585] [11] [*see BMB* 578]

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THE CHEMICAL NATURE OF BACTERIAL ANTIGENS

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Our present knowledge of the chemical nature of the somatic antigens of bacteria originated in the first place from the results obtained by applying immunological techniques, which were elaborated during the early part of the present century, for distinguishing between the antigenic components of various species of bacteria. The isolation and identification of these antigenic complexes was undertaken as soon as their qualitative detection and quantitative measurement had been established by these techniques.

Pick (1912) observed that typhoid cultures that had been rendered protein-free by treating them with trypsin and pepsin, and by subsequent boiling to remove soluble protein, contained a substance which formed a precipitate in the presence of typhoid immune-serum. Similar observations were recorded by Dochez & Avery (1917) for *Pneumococcus*, and by Zinsser (1921) who worked with *Pneumococcus*, *Staphylococcus* and the typhoid bacillus. Somewhat later, serologically-active substances were isolated by chemical procedures from each of the main types of *Pneumococcus*, and were identified as complex polysaccharides which are located in the bacterial capsules of the different strains (Heidelberger & Avery, 1923, 1924; Avery & Goebel, 1933; Heidelberger, Kendall & Scherp, 1936; Brown, 1939; Brown & Robinson, 1943). The methods used in the investigations have been extended to many pathogenic and non-pathogenic strains of bacteria in the Gram-positive and Gram-negative groups.

In almost every instance the purified specific-soluble substance was found to lack significant antigenic properties, as it failed to induce the formation of a type-specific precipitin, agglutinin or protective antibody in the serum of rabbits that had received intravenous injections of polysaccharide material. As a result of this work, a conception of antigenicity which attributed the specific immunological properties of a complete bacterial somatic antigen to a polysaccharide constituent, and the antigenic properties of the complete bacterial complex to a combination of this important constituent with some other chemical component, came to be generally accepted and led to the idea that the difference between a virulent and an avirulent strain of the same organism is the presence in the former of the specific antigenic complex, which is not found in the avirulent form. Substances, such as the specific polysaccharides, that do not possess complete antigenic power but have the capacity of reacting *in vitro* with the immune-sera prepared by using the whole antigen, have been termed haptens by Landsteiner.

In the space available here it is not possible to deal in detail with the chemical nature of the large number of specific bacterial somatic substances that have been described. The account that follows is, therefore, limited to a description of one or two examples of typical haptens and antigens selected from the groups of Gram-positive and Gram-negative organisms. The chemical nature of the so-called bacterial toxins and the antigenic complexes of animal and plant viruses are not discussed.

Specific Components of the Antigenic Complexes of Gram-positive Bacteria

The polysaccharide components of the type-specific antigens of the pneumococci were among the first bacterial haptens to be isolated and identified, and, of these biologically important complex molecules, our knowledge of the specific polysaccharide of *Pneumococcus* Type III is more complete than that of any other bacterial polysaccharide. The polysaccharide of *Pneumococcus* Type III is broken down by acid hydrolysis into aldobionic-acid units, each composed of one molecule of glucose and one molecule of glucuronic acid, united by a glycoside link involving the reducing (aldehyde) group of the glucuronic acid and carbon atom 4 of the glucose molecule. The aldobionic acid molecules are likewise joined together by a glycoside link which involves the reducing group of the glucose component and the hydroxyl group attached to the third carbon atom in the glucuronic acid molecule. According to Reeves & Goebel (1941) the polysaccharide molecule may be formulated as

-4 glucose-1 : 3 glucuronic acid-1 : 4 glucose-

During work on the serological differentiation of the pneumococcal types, it had been observed that there was a considerable degree of overlapping in the specific reactions of Types III and VIII of the pneumococcus. With the isolation and subsequent examination of the specific polysaccharide from the Type VIII organisms by Goebel (1935), it became evident that the specific polysaccharide of each of these types contains a common aldobionic acid component, and thus it would appear that it is this component which is responsible for their overlapping specificity. Determination of the proportions of the constituent molecules in each of the two polysaccharides revealed that the Type VIII polysaccharide contained glucose units approximating to the ratio of 7 molecules of glucose to 2 of glucuronic acid, as compared with a 1 : 1 ratio for the Type III polysaccharide.

Avery & Goebel (1933) showed that the component responsible for the type-specific reactions of *Pneumococcus* Type I is an acetylpolysaccharide which possesses acidic characters, contains 6·0% of acetyl groups and 4·8% N, about a third of which is probably present as a primary amino group. The substance is strongly dextro-rotatory, $[\alpha]_D + 255°$, and gives rise to 32% of reducing sugar on acid hydrolysis. This polysaccharide was found to possess the unusual property of inducing the formation of protective antibody. Thus mice, when injected intraperitoneally with minute quantities of the acetylpolysaccharide, responded with an active immunity to subsequent infection with the homologous organism. On the other hand, no type-specific precipitins, agglutinins or protective antibodies were demonstrable in the serum of rabbits that had received repeated intravenous injections of the Type I acetylpolysaccharide. These experiments revealed, therefore, points of difference between the antigenic properties of the acetylated polysaccharide and those of the type-specific antigen of the intact virulent coccus, and indicated that the native type-specific antigen had not been isolated. The acetylated polysaccharide is clearly an important component of the type-specific antigenic complex, but it does not possess the full antigenicity of the naturally-occurring antigen.

The kind of substance with which the specific polysaccharides in the different types of pneumococci are combined to render them fully antigenic and thus able to induce the formation of the homologous type-specific immune-sera, is not known with certainty. If, as appears probable, the proteins that are present in all types of pneumococci are similar, then, in the light of some recent observations with

other antigenic complexes (see Morgan & Partridge, 1941; Morgan, 1943a; 1943b), it may be anticipated that the specific polysaccharide-protein combination will prove to be antigenic and will give rise to an immune-body that is specific for the polysaccharide component of the complex, and thus specific for the pneumococcal type from which the polysaccharide had been isolated. But it is doubtful whether, up to the present time, there is any record of the isolation and identification of a complete type-specific somatic antigen that is associated with a capsular polysaccharide of a Gram-positive organism.

The antigenic analysis of haemolytic streptococci has revealed a different type of antigenic complex, for the type-specific properties reside in a protein substance, M, and in another unidentified type-specific compound T (Lancefield, 1940; Zittle, 1942). The M protein can be isolated from the virulent organism by extraction with 0·05 *N* HCl and purified by salt fractionation, iso-electric precipitation, and dialysis. The species-specific properties of most of the strains of human origin (Group A) are due to a polysaccharide which contains 1·7 % N, yields 87 % of reducing substances after acid hydrolysis and possesses a strong *laevo* rotation (Zittle & Harris, 1941). The capsular substance of the haemolytic streptococci consists largely of hyaluronic acid, a polysaccharide built up from molecules of glucuronic acid and N-acetylglucosamine (Kendall, Heidelberger & Dawson, 1937). The capsular material in this instance appears to play no part in the specific serological reactions of the haemolytic streptococci and cannot therefore be considered to be the specific hapten in spite of its location in the bacterial capsule. It seems, however, to be a factor of definite significance in the virulence or invasiveness of these organisms (Seastone, 1943).

The specific capsular substance of virulent strains of the anthrax bacillus is not composed of a polysaccharide, as for most organisms, but consists of a protein-like substance which is composed of d(-) glutamic acid molecules linked together to form a long-chain polypeptide (Ivánovics & Bruchner, 1937; Ivánovics, 1940). The substance gives precipitation with the sera of rabbits hyper-immunized with killed encapsulated anthrax bacilli; and such sera can protect mice against anthrax infection. The capsular material is built up from the " unnatural " form of glutamic acid, and the suggestion has been made that the resistance of this substance to the action of the tissue-enzymes is most probably responsible for the virulence of the encapsulated form of the organism. The somatic substance of both the virulent and avirulent bacillus contains an antigen, the specificity of which is determined by a nitrogenous polysaccharide, composed of *d*-galactose and *d*-glucosamine.

Type-specific polysaccharides have been obtained from *Staphylococcus*, Types A and B, by Wieghard & Julianelle (1935), but the corresponding antigenic complex has not been isolated. The more recent work of Verwey (1940) suggests that there is also present a type-specific antigenic protein. This material reacts in high dilution with homologous immune-sera prepared by injecting animals with whole organisms or with the protein substance itself.

Isolation of Specific Somatic Antigens from Gram-negative Bacteria

The chemical nature of the somatic antigens of the Gram-negative bacteria has in some instances been investigated in considerable detail. Indeed, careful study of the antigens of the dysentery-typhoid group of organisms has revealed much of the inner structure of these complex molecules and has shown that in many biologically unrelated organisms the general make-up of the characteristic somatic antigen is very similar.

Until about 10 years ago attempts to separate the somatic antigens from bacteria were unsuccessful, but in 1933 Boivin and Mesrobeanu devised a simple technique whereby antigenic material could be obtained from Gram-negative organisms by extracting the wet bacteria with dilute trichloracetic acid (*p*H 1·0–2·0) at 0° C. The antigenic complex passed into solution and was freed from bacterial bodies by centrifugation and filtration. About the same time, and quite independently, Raistrick & Topley (1934) found that by the action of trypsin on *Bact. aertrycke* it was possible to bring the specific somatic antigenic complex into solution. The digestion was carried out at *p*H 8·3–8·5 for several days at 37° C. Freeman, Challinor & Wilson (1940), using the same method of isolation, extended these observations, compared the properties of antigenic material isolated from organisms that had been grown on ordinary and synthetic media, and concluded that the essential antigenic complex could be obtained in a state approaching chemical homogeneity from cultures grown on either medium.

A method for the isolation of bacterial antigens from Gram-negative organisms which avoids the use of an acid or alkaline medium, and which can be carried out at a low temperature, was introduced by Morgan (1937) in order to minimize the risk of inducing changes in the antigenic complex during the process of isolation. The extraction process was based on the solubility of the antigenic material in water-soluble organic solvents, anhydrous diethyleneglycol being the one most extensively used. The use of a solvent of this kind allows the antigen to be separated from 90–95 % of the total material of the bacterial bodies and thus renders its subsequent isolation from the non-specific somatic substances relatively easy. Furthermore, the use of anhydrous organic solvents reduces the possibility of extracting enzymes from the bacterial bodies that are destructive to the antigen by subsequently attacking the antigenic complex when, at a later stage in its purification, it is obtained in aqueous solution.

Chemical Nature of the Somatic Antigens of Gram-negative Organisms

The first insight into the nature of the specific somatic antigens of Gram-negative bacteria was obtained by Boivin & Mesrobeanu (1933), who showed that the material extracted by trichloracetic acid from certain strains of *Salmonella* could be split by gentle acid hydrolysis into a polysaccharide that was specific for each specimen examined, and a fatty or phospholipin component which possessed no obvious immunological property. In a long series of papers, Boivin and his co-workers (see Boivin & Mesrobeanu, 1935; Mesrobeanu, 1936; Soru & Combiesco, 1940) showed that the polysaccharide-phospholipin type of antigenic complex was present in many different species of Gram-negative bacilli. The complexes were found to be toxic for animals and were believed to be free from protein. Raistrick & Topley (1934) considered that, from the evidence then available, a final conclusion as to the composition of the antigenic complex could not be reached, other than that it contained the specific polysaccharide in an antigenic form. Topley, Raistrick, Wilson, Stacey, Challinor & Clark (1937) subsequently concluded that the antigenic material isolated by means of tryptic digestion of *Bact. aertrycke* and *Bact. typhosum* was similar to that isolated by Boivin by means of trichloracetic acid. These workers examined antigenic extracts that contained more than one type of antigen, and showed that certain qualitative tests, such as the formation of a precipitate with aluminium sulphate or uranium acetate, could indicate the presence of the " virulence " or " Vi " antigen (Felix & Pitt, 1934) in material isolated from strains of *Bact. typhosum* which contained this antigen in addition to the characteristic O-somatic antigen. They also pointed out that the O-antigen in the presence of Vi-antigen was much more resistant to hydrolysis by 0·1 *N* acetic acid at 100° C. than the O-antigen alone.

Morgan (1937), Henderson & Morgan (1938) and Morgan & Partridge (1939, 1940, 1942) considered that the O-somatic material extracted by diethyleneglycol from *Bact. dysenteriae* (Shiga) and *Bact. typhosum* was a homogeneous molecular complex containing a polypeptide-like or protein component, a specific polysaccharide and a phospholipin complex. The degradation of these antigenic complexes by means of anhydrous formamide was studied in considerable detail and it was shown for the antigen of *Bact. dysenteriae* (Shiga) that the phospholipin ($[\alpha]_{5461} + 12°$; N, 1·8 %; P, 3·9), although more or less firmly bound to the other components, could be removed by means of the dissociating action of formamide without inducing a significant change in the immunological properties of the antigen. Thus the phospholipin-free material was found to be fully antigenic, and induced in rabbits the production of specific agglutinins and precipitins and the " Shiga " heterophile immune-body. It appears, therefore, that the main phospholipin component of the complex is not essential for the manifestation of antigenic properties. More thorough treatment with formamide gives rise to complete dissociation of the remaining polysaccharide-protein complex, and it was possible by this means to obtain for the first time a specific polysaccharide in its native or undegraded

condition. The polysaccharide obtained by this method ($[\alpha]_{5461} + 85°$; N, 1·7%) is highly viscous in aqueous solution, contains a significant amount of organic phosphorus (0·5–1%), but fails to induce the formation of specific agglutinins, precipitins or protective immune-body in the rabbit. The observation was made that preparations of the polysaccharide in which only a few per cent. of the protein component ($[\alpha]_{5461} - 48°$; N, 11·5–12·5%, P, 0·8–1·0%) remain are quite strong antigens, and it is essential, therefore, to establish beyond doubt that bacterial polysaccharide preparations are completely free from protein before concluding that the specific polysaccharides themselves are antigenic. The action of trypsin on the complete antigen has made it possible to isolate the phospholipin-polysaccharide part of the complex; this was found to be without significant antigenic properties. Similar observations were made by Freeman & Anderson (1941) for *Bact. typhosum*.

There is evidence which indicates that the native antigenic complex, as it exists in the intact bacterial cell, contains substances other than the phospholipin, polysaccharide and protein components, but it would appear from the dissociation experiments just described that, for some somatic antigens at least, the protein and the polysaccharide components are the two constituents whose presence is essential if the resulting complex is to possess the power to give rise to an immune-body specific for its polysaccharide component.

By utilizing the action of dilute alkali or 90% phenol on the native phospholipin-polysaccharide-protein antigen isolated from *Bact. dysenteriae* (Shiga) and *Bact. typhosum*, Morgan & Partridge (1941, 1942) obtained evidence that the protein component, which is toxic for animals, contains a prosthetic group and is, therefore, a conjugated protein. A similar conclusion has been reached by Freeman (1943), who has obtained a protein substance from the O-somatic antigen of *Bact. typhi-murium*. Observations (Morgan, unpublished) of the same kind have been made for the composition of the whole antigen complex and for the protein component of the O-somatic antigens of the various types of *Bact. dysenteriae* (Flexner). The true nature of the prosthetic group is not known. These results indicate that certain groups of bacteria possess somatic antigens which are built up from similar types of molecules arranged together as polymolecular aggregates. According to Morgan & Partridge (1940), each antigenic complex consists of a polysaccharide that is specific for the organism and responsible for its characteristic serological reactions, and a conjugated protein component which endows the polysaccharide with antigenic properties. Other components of the native antigenic complex such as the phospholipin, are also present but are not essential for the manifestation of antigenicity. Further unidentified substances are possibly also present in the native antigen.

Considerable support for this conception of antigenic structure was obtained when it was observed by Partridge & Morgan (1940) that the isolated, undegraded and non-antigenic specific polysaccharide component of the O-antigen of *Bact. dysenteriae* (Shiga) could be re-combined with the non-specific conjugated protein of the native antigen to yield an antigenic complex that would give rise to antibodies which are specific for the polysaccharide component, and thus for the homologous organism. It was also found that specific somatic polysaccharides obtained from organisms belonging to the dysentery-typhoid group combine readily with the conjugated protein component derived from the corresponding somatic antigen of another organism within the group, and that the artificial antigen so formed induces the formation of a specific immune-body for the polysaccharide component. Further evidence which supports these views on antigenic composition is provided by the success with which certain non-antigenic polysaccharides, such as agar, gum acacia, and cherry gum (Partridge & Morgan, 1942) or the specific blood-group A or B haptens of animal or human origin (Morgan, 1943a, 1943b; Morgan & King, 1943; King & Morgan, 1944 and unpublished observations) have been converted to full antigens by combination with the reactive bacterial conjugated protein mentioned above.

The polysaccharide component of the "Shiga" antigen contains *d*-galactose, *l*-rhamnose and N-acetylhexosamine (Morgan, 1938) whereas the corresponding hapten from *Bact. typhosum* is built up from molecules of *d*-glucose, *d*-mannose and *d*-galactose (Freeman, 1942). The main phospholipin component of the O-antigen of *Bact. dysenteriae* (Shiga) yields α-glycerophosphoric acid, oleic acid and palmitic acid on saponification with alcoholic potash, and is similar in certain of its properties to kephalin rather than lecithin (Morgan & Partridge, 1940).

The nature of the antigenic complex of another Gram-negative organism, *Br. melitensis*, has been considered in detail by Miles & Pirie (1939), who have employed a different method of approach from those already described. Suspensions of *Br. melitensis* in chloroform water or dilute phenol (2%) were found to give up their specific antigen, which could be recovered from solution by differential sedimentation. Further fractionation of the material with ammonium sulphate gave rise to viscous opalescent solutions of the material that showed anisotropy of flow. Certain lipoid components of the complex were readily removed by treatment with organic solvents and protein-like material was eliminated by treatment with acetic acid. Gentle hydrolysis of the antigen gave rise to a formyl derivative of an amino-polyhydroxy compound and an insoluble phospholipin. The formyl derivative of the polyhydroxy compound is an inhibitory hapten, but loses this power with the disappearance of a phospholipin group, on further hydrolysis; this component is neither toxic nor antigenic. Preparations of the antigen in different states of agregation differ in antigenicity, toxicity and immunizing power.

General Conclusions

The steady increase in our knowledge of the chemical nature of bacterial antigens is likely to lead to the preparation of these biologically important complexes in a form which is free from non-specific substances and therefore more suitable for prophylactic immunization than the crude bacterial suspensions that have so far been employed. A beginning has already been made, and the extracted and purified O-somatic antigen of *Bact. dysenteriae* has been employed for the immunization of man against infection with this organism (Morgan & Schütze, 1943). Ultimately, when the exact nature and function of the component parts of antigens that have been derived from pathogenic bacteria are known, it may be possible to synthesize, by the methods of organic chemistry, hapten molecules of relatively simple composition. These might be combined with a suitable protein component and used to induce the formation of immune-bodies that will function therapeutically with an efficiency equal to the more highly specific antibodies that are produced by immunizing with whole organisms. Progress along these lines has also been made by Goebel (1939). The old adage "prevention is better than cure" is surely the right approach to the problem of the control of infectious disease, and we may anticipate with confidence the successful use of purified preparations of natural antigens or of artificial antigenic complexes built up from natural or synthetic haptens for the purpose of establishing an effective immunity in man.

References

[*Full references to the earlier work on the chemical and immunological properties of the specific bacterial polysaccharides and antigenic analysis are contained in Landsteiner, K.* (1936) "*The specificity of serological reactions*," *Baltimore; Marrack, J. R.* (1938) "*The chemistry of antigens and antibodies*," *London; Topley, W. W. C. & Wilson, G. S.* (1936) "*The principles of bacteriology and immunity*," *London*]

Avery, O. T. & Goebel, W. F. (1933) *J. exp. Med.* **58**, 731
Boivin, A. & Mesrobeanu, L. (1933) *C.R. Soc. Biol. Paris*, **112**, 76
Boivin, A. & Mesrobeanu, L. (1935) *Rev. Immunol.* **1**, 553
Brown, R. (1939) *J. Immunol.* **37**, 445
Brown, R. & Robinson, L. K. (1943) *J. Immunol.* **47**, 7
Dochez, A. R. & Avery, O. T. (1917) *J. exp. Med.* **26**, 477
Felix, A. & Pitt, R. M. (1934) *Lancet*, **2**, 186
Freeman, G. G. (1942) *Biochem. J.* **36**, 340
Freeman, G. G. (1943) *Biochem. J.* **37**, 601
Freeman, G. G., Challinor, S. W. & Wilson, J. (1940) *Biochem. J.* **34**, 307
Freeman, G. G. & Anderson, T. H. (1941) *Biochem. J.* **35**, 564
Goebel, W. F. (1935) *J. biol. Chem.* **110**, 391
Goebel, W. F. (1939) *J. exp. Med.* **69**, 353
Heidelberger, M. & Avery, O. T. (1923) *J. exp. Med.* **38**, 73
Heidelberger, M. & Avery, O. T. (1924) *J. exp. Med.* **40**, 301

Heidelberger, M., Kendall, F. E. & Scherp, H. W. (1936) *J. exp. Med.* **64**, 559
Henderson, D. W. & Morgan, W. T. J. (1938) *Brit. J. exp. Path.* **19**, 82
Ivánovics, G. (1940) *Z. ImmunForsch.* **97**, 402
Ivánovics, G. & Bruckner, V. (1937) *Z. ImmunForsch.* **93**, 119
Kendall, F. E., Heidelberger, M. & Dawson, M. H. (1937) *J. biol. Chem.* **118**, 61
King, H. K. & Morgan, W. T. J. (1944) *Biochem. J.* **38**, x
Lancefield, R. C. (1940) *J. exp. Med.* **71**, 521
Mesrobeanu, L. (1936) *Arch. roum. Path. exp. Microbiol.* **9**, 1
Miles, A. A. & Pirie, N. W. (1939) *Brit. J. exp. Path.* **20**, 83, 109 & 278
Miles, A. A. & Pirie, N. W. (1939) *Biochem. J.* **33**, 1097 & 1716
Morgan, W. T. J. (1937) *Biochem. J.* **31**, 2003
Morgan, W. T. J. (1938) *Helv. chim. Acta*, **21**, 469
Morgan, W. T. J. (1943a) *Nature, Lond.* **152**, 82
Morgan, W. T. J. (1943b) *Brit. J. exp. Path.* **24**, 41
Morgan, W. T. J. & King, H. K. (1943) *Biochem. J.* **37**, 640
Morgan, W. T. J. & Partridge, S. M. (1939) *Nature, Lond.* **143**, 1025
Morgan, W. T. J. & Partridge, S. M. (1940) *Biochem. J.* **34**, 169
Morgan, W. T. J. & Partridge, S. M. (1941) *Biochem. J.* **35**, 1140
Morgan, W. T. J. & Partridge, S. M. (1942) *Brit. J. exp. Path.* **23**, 151
Morgan, W. T. J. & Schütze, H. (1943) *Lancet*, **2**, 284
Partridge, S. M. & Morgan, W. T. J. (1940) *Brit. J. exp. Path.* **21**, 180
Partridge, S. M. & Morgan, W. T. J. (1942) *Brit. J. exp. Path.* **23**, 84
Pick, E. P. (1912) In *Kolle und Wassermann's "Handbuch der pathogenen Mikroorganismen,"* Jena
Reeves, R. E. & Goebel, W. F. (1941) *J. biol. Chem.* **139**, 511
Raistrick, H. & Topley, W. W. C. (1934) *Brit. J. exp. Path.* **15**, 113
Seastone, C. V. (1943) *J. exp. Med.* **77**, 21
Soru, E. & Combiesco, D. (1940) *C.R. Soc. Biol. Paris*, **133**, 498
Topley, W. W. C., Raistrick, H., Wilson, J., Stacey, M., Challinor, S. W. & Clark, R. O. J. (1937) *Lancet*, **1**, 252
Verwey, W. F. (1940) *J. exp. Med.* **71**, 635
Wieghard, C. W. & Julianelle, L. A. (1935) *J. exp. Med.* **62**, 23 & 31
Zinsser, H. (1921) *J. exp. Med.* **34**, 495
Zittle, C. A. (1942) *J. Immunol.* **43**, 31
Zittle, C. A. & Harris, T. N. (1941) *J. biol. Chem.* **142**, 823

THE NATIONAL COLLECTION OF TYPE CULTURES

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With the insistent growth of microbiology from the pioneer days of Pasteur and Koch and the " Golden Age " of bacteriology, the need for type-culture collections of micro-organisms has continually been present in the minds of workers in the various fields, both for the study of principles and methods in microbiological investigation, and for the systematic classification of bacteria in their various species and strains.

Before the war of 1914–18, the principal sources of supply available for microbiologists were the collection of the American Museum of Natural History in New York, the Kral Collection in Vienna and the Collection of the Pasteur Institute in Paris. In addition to these, there were what might be called specialist collections, mostly on the continent of Europe, such as the *Centraal-bureau voor Schimmelcultures* at Baarn, Holland, and the associated *Laboratorium voor Microbiologie* at Delft, the Collection of the *Carlsberg Laboratorium* at Valby, near Copenhagen, which concerned itself with the maintenance of yeasts, and the Collection of the *Institut für Gärungsgewerbe* in Berlin. It is a matter for regret that the Carlsberg and Kral Collections have disappeared under the stress of economic pressure. The American Type Culture Collection, enlarged and reconstituted, is at present domiciled at the Georgetown University Medical School in Washington, D.C. under the curatorship of Professor Mario Mollari. In Rio de Janeiro a noteworthy collection of micro-fungi is maintained by Dr. O. da Fonseca at the *Instituto Oswaldo Cruz*.

Foundation of the Collection

In the early years of the present century there was no representative collection of micro-organisms in Britain, but in the year 1920 the *National Collection of Type Cultures* was founded under the joint control of the *Medical Research Council* and the *Lister* Institute of Preventive Medicine, who had long contemplated the formation of such a collection from which microbiologists in general and bacteriologists in particular might obtain from a trustworthy source authentic strains of recognized micro-organisms for use in scientific work. The *Lister* Institute provided, and continues to provide, accommodation and the necessary amenities, while the *Medical Research Council* bears the expenses of administration and of any special equipment that may from time to time become necessary.

The unit was formed under the general direction of the late Sir John Ledingham, F.R.S., then Chief Bacteriologist to the *Lister* Institute. He retired from the directorship of the Collection in 1930 on his appointment as Director of the *Lister* Institute, since when the Curator—the author of this article—has acted as chief executive officer.

The nucleus of the Collection was formed from the private collection of the *Lister* Institute of some few hundred strains, which included the valuable collection of anaerobic bacteria (*Clostridia*) of Dr. Muriel Robertson, and the yeast collection of the Nobel Laureate—the late Professor Sir Arthur Harden, F.R.S. It has grown steadily from year to year, and has been enriched by donations from many sources. Among these may be mentioned over 150 separate species or subspecies of food-poisoning organisms belonging to the genus *Salmonella* received from Dr. H. Schütze (*Lister* Institute), Dr. Fritz Kauffmann (University of Lund, Sweden) and Dr. P. R. Edwards (Agricultural Experiment Station, Lexington, Kentucky, U.S.A.); *Aspergillus* and *Penicillium* species from Dr. Charles Thom and Dr. Kenneth Raper (U.S. Department of Agriculture) and from Professor H. Raistrick, F.R.S. (London School of Hygiene); collections of sporing aerobic bacteria (*Bacillus*) from Dr. W. W. Ford (Johns Hopkins University, Baltimore), Dr. Nathan Smith (Department of Agriculture, U.S.A.) and Dr. T. Gibson (Edinburgh and East of Scotland College of Agriculture); pathogenic fungi of interest in medical mycology from Sabouraud's collection through Dr. J. T. Duncan (London School of Hygiene) and from Dr. Roger Baker (Duke University School of Medicine, North Carolina, U.S.A.); grouped strain of *Pasteurella* from Dr. J. T. Cornelius, and of *Bacterium pseudotuberculosis-rodentium* from Dr. H. Schütze; a collection of viruses of human and animal origin from Dr. E. W. Hurst (*Lister* Institute); *Streptococcus pyogenes* types from the late Dr. F. Griffith (Ministry of Health); American pneumococcus types from Dr. R. S. Muckenfuss (Rockefeller Institute, New York) and English types from Dr. M. Y. Young (*St. Mary's* Hospital) through Sir Alexander Fleming, F.R.S.; soil organisms from Dr. H. G. Thornton, F.R.S. (Rothamsted Experimental Station) and Dr. S. A. Waksman (Agricultural Experiment Station, New Jersey, U.S.A.) and representatives of Gardner and Venkatraman's *Vibrio* groups I to VI, from Professor A. D. Gardner (School of Pathology, Oxford).

Work and Personnel

In the first year of the Collection's existence over 1,500 cultures were distributed to workers at home and abroad. This figure increased steadily from year to year, so that by the last academic year before the present war the number sent to correspondents throughout the world had reached a total of over 6,400 strains. Of these some 45 % were issued for purposes of scientific research, 20 % to colleges and schools for teaching purposes, and 35 % in connection with technology. The Collection now comprises some 4,000 strains of micro-organisms, consisting of about 1,400 separate species. Of these about half are bacteria—the remainder fungi, yeasts and associated organisms. Large numbers of strains of micro-organisms are lodged year by year for purposes of maintenance or investiga-

tion. The struggle in which we are still involved has sadly curtailed overseas activities, as can well be imagined, but the demand for cultures in the British Isles has, apparently, been stimulated by war conditions and the staff has been very fully occupied, in spite of temporary isolation from many European and other colleagues.

The personnel of the Collection consists of a Curator, Assistant Curator and technician with the necessary laboratory assistants—the whole under the general direction of the *Medical Research Council*, of which the present Director of the Lister Institute—Dr. Alan N. Drury, F.R.S.—is a member. The Collection is a general one, but plans are in the making which will to some extent modify its structure in the post-war years and make it more adequate to serve the special needs of research workers in medical and veterinary bacteriology. Nevertheless, though at the present time the medical and veterinary aspects of the Collection are particularly stressed, the needs of agriculture, technology and teaching are well catered for—indeed the Collection on its present basis is of service to all workers in the various disciplines of microbiology.

The Collection also acts as a useful repository for important strains, which the original investigator, for one reason or another, cannot maintain. Examples of these in the National Collection are numerous yeast cultures formerly preserved at the *Carlsberg Laboratorium*; a collection of *Actinomyces* species from Jamaica, which are antagonistic to the growth of *Fusarium oxysporum* var. *cubense*—the causal organism of Panama disease of bananas; as also a series of organisms (*Acetobacter*) isolated from vinegar brews in East Africa. These examples serve to indicate the varied material with which the Collection has to deal and the diversity of places from which it originates. Attention is directed to the continued need for the deposition of newly described species and also for fresh examples of familiar types. It is only by constant augmentation and replenishment of such material that the requirements of workers at home and abroad in the various disciplines of microbiology can adequately be satisfied. The co-operation of microbiologists is earnestly invited and in return every effort is made to supply the needs of applicants for cultures. The staff is always glad to help correspondents in the identification of material and cultures, which should be accompanied by the fullest particulars as to source and date of isolation and, if possible, by clinical and epidemiological notes. Cultures are supplied on demand, so far as possible, to workers throughout the world. A small charge is usually made to help to defray the cost of materials and postage.

A new edition of the Catalogue of the National Collection is at present in preparation and will be issued as soon as circumstances permit after the war. In addition to the catalogue, a list of fungi maintained in the Collection is printed from time to time in the *Transactions of the British Mycological Society*. The staff of the Collection is in close touch with widespread activities. For example, the Curator has acted in an executive capacity at the Paris, London and New York Congresses for Microbiology and is at present serving as Secretary of the Permanent International Commission, the Nomenclature Commission and the Salmonella Sub-committee of the International Association of Microbiologists. The staff, in addition, is actively interested in problems of medical mycology and in the formulation of the proposed rules of bacteriological classification.

In addition to the routine subculture of numbers of strains, on appropriate media, cultures are also kept as desiccates, whenever possible, thereby preserving their biological characters unaltered for many years. For some considerable time a simple but effective technique of "snap-freezing", devised by Professor Alfredo Sordelli of Buenos Aires, has been employed. This method is of value in the preservation of small quantities of material. It consists of rapid evacuation with a Hyvac pump, trapped with phosphorus pentoxide, avoiding the lethal effects of concentration and aggregation of protein molecules in the process of desiccation, which leads to their denaturation. The necessity of employing a special freezing agent, such as "dry ice", is thus eliminated.

All organisms listed in the catalogue of the Collection are maintained at the *Lister* Institute, Elstree, Hertfordshire, where the Collection was removed, from London, on the outbreak of war, with the exception of fungi causing disease or spoilage of timber, which are the special care of the Forest Products Research Laboratory at Princes Risborough, and most of the filterable viruses and bacteriophages, which are obtainable in certain cases from specialists in the various groups. Thus a start has been made in a process of decentralization which will be referred to later.

Future Development

With regard to the general question of the future of type-culture collections, two lines of development are possible. The maintenance of large general collections presents increasing difficulties of administration and, with ever-expanding knowledge in the various fields of microbiology, it will become more and more difficult to maintain such collections under the direction of a small, even if devoted, band of workers. Such a staff may, and indeed often does, possess a wide knowledge of microbiology in all its aspects, but cannot possess intimate specialized knowledge of all the groups comprised in a large general collection; nor are the laboratory facilities usually sufficient for the extensive and minute examination and periodic overhaul of such an expanding collection. Short of an Institute of Microbiology, with ample funds, staff and accommodation, there is not much hope for expansion in this direction. Nevertheless the idea is not without attraction. The growing interdependence of the various branches of microbiology has been stressed recently in Britain by the formation of a new Society—The Society for General Microbiology—which has received influential support from every field of microbiology.

A second line of development is more immediately hopeful. In place of the large general collection, kept to serve the ordinary requirements of microbiologists, it is suggested that a greater measure of decentralization than at present employed should be introduced. Responsibility in respect of a number of groups of organisms might be delegated to appropriate specialized reference laboratories (many of which already exist in connection with the Emergency Public Health Laboratory Service formed under the aegis of the *Medical Research Council*), while a limited selection of "tested cultures" for use in medical and veterinary teaching would be kept under constant supervision at headquarters. While retaining a nucleus to meet the needs of routine and teaching purposes, the present collection might well act as a central bureau or clearing house and be in close association with specialist correspondents in the various groups who would undertake to supply cultures on request. Such a system need not be costly, but would require much careful organization.

International Collaboration

To take the broader aspect of international collaboration and reciprocity one may envisage at some future date the formation of an International Commission or International Advisory Committee on Culture Collections, sponsored in all probability by the International Association of Microbiologists, whose activities, exemplified by its Paris, London and New York Congresses, have unfortunately been interrupted by the war. Much good work in the international sphere has been delayed by the impossibility of holding the Fourth International Congress in Copenhagen in 1942, as arranged. The international character of such an organization might be developed through official and unofficial agencies in the different countries concerned, the objects of such an organization being briefly as follows:

To make more adequate provision for permanent reservoirs of collections of living micro-organisms (including bacteria, protozoa, micro-fungi, micro-algae and viruses), particularly those of importance in medical, veterinary, agricultural and industrial science.

To provide more effectively for the needs of workers in fundamental biological studies, such as genetics, cellular physiology and biochemistry.

To develop new facilities in continental areas not at present adequately served and to expand the scope and utility of existing culture collections.

To promote international co-operation and integration in these respects through appropriate channels.

It is becoming increasingly evident that international scientific co-operation has received an immense stimulus as a result of the pooling of information during the present war. Moreover, increased facilities for rapid communication provided by modern air transport will, in the future, greatly expedite the interchange of scientific material and information. In this dissemination the various type-culture collections will

surely take part—an immediate need to be filled being the share they will take in the scientific rehabilitation of the countries that have been ravaged and impoverished by war, by supplying type cultures for comparison with others and for fundamental scientific research.

It appears, therefore, expedient to have some kind of "blueprint," as outlined above, in readiness for the days to come. If in the future a scheme of this character should be evolved, it would, without doubt, help to promote mutual good will and understanding among the best elements of the various nations, and contribute effectively to that reciprocity in microbiological science which is so greatly to be desired.

REVIEW OF SELECTED PAPERS

Influenza

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STUDIES ON AIR-BORNE VIRUS INFECTIONS. III. The Killing of Aerial Suspensions of Influenza Virus by Hypochlorous Acid

by D. G. ff. Edward & O. M. Lidwell, *Journal of Hygiene*, **43**, 196–200, September 1943

The possibility of destroying influenza virus by aerial disinfection is perhaps of particular interest at the present time. It has been found by Andrewes (1940) that aerosols of influenza virus could be rendered non-infective by sodium hypochlorite mists; Drs. Edward and Lidwell, also working at the National Institute for Medical Research in London, have now given details of experimental findings. The influenza-virus suspensions used were prepared from the lungs of mice which had been infected with the PR8 and W.S. strains. In 4 of 5 experiments in which virus aerosols were in contact with hypochlorous acid gas in concentrations of from 1 : 640,000 to 1 : 3,000,000 for 20 minutes, 99 % of the virus was destroyed. The virus aerosol was as easily killed by the gas as *Streptococcus salivarius*. Preliminary experiments on mice and cats failed to show any toxic effects produced by inhaling the gas in relatively high concentration for prolonged periods.

REFERENCE

Andrewes, C. H. (1940) *Lancet*, **2**, 770

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CLINICAL STUDY OF AN OUTBREAK OF INFLUENZA B DURING THE WINTER, 1942–43

by J. M. Stansfeld & C. H. Stuart-Harris, *Lancet*, **2**, 789–790, 25/12/43

This paper records the clinical data of the outbreak described by Stuart-Harris, Glover & Mills (1943). The patients came from certain military units and had been admitted to camp reception stations with fever and acute respiratory infections, but not all were considered typical influenza. Records of 25 cases in an American hospital (in Britain) suffering from influenza-like illness were also available. Hirst serum tests were made on both acute and convalescent sera of all patients, and according to the rise of antibody to influenza B they were placed in three categories: (i) A significant (4-fold or greater) rise of antibody was obtained in 24 cases from the reception stations and in 11 cases from the American hospital; (ii) no rise in antibody could be detected in 14 patients from the reception stations, nor in 12 from the American hospital; (iii) four cases from the reception stations and 2 from the American hospital gave a 2- to 3-fold rise in titre. Sera from all cases in categories (ii) and (iii) and from 6 cases in category (i) were tested against virus A, but with negative results. The signs and symptoms of 24 of the cases of influenza B and 12 cases of influenza Y (those whose type of influenza was not determined serologically) were then compared with the signs and symptoms of 60 cases of influenza A studied during 1937–41. In addition to clinical data, radiological findings were available in 20 cases, and leucocyte counts in all 25 cases in the American hospital.

The conclusion reached from a comparison of cases of influenza A, B and Y was that the three differed little in the incidence of general aching or coryza, although the average maximum temperature was a little lower in the B group. It has been suggested by Taylor, Parodi, Fernandez & Chialvo (1942) that clinical differences may be detectable between influenza caused by virus A and by virus B, but the present authors found that the two groups were indistinguishable.

REFERENCES

[1] Stuart-Harris, C. H., Glover, R. E. & Mills, K. C. (1943) *Lancet*, **2**, 790

Taylor, R. M., Parodi, A. S., Fernandez, R. B. & Chialvo, R. J. (1942) *Rev. Inst. bact. C. G. Malbrán*, **2**, 44

[1] [*see BMB* **559**]

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INFLUENZA IN BRITAIN, 1942–43

by C. H. Stuart-Harris, R. E. Glover & K. C. Mills, *Lancet*, **2**, 790–793, 25/12/43

During the winter and spring of 1941–42 and 1942–43 workers at the National Institute for Medical Research, London, and at a military laboratory, have continued to study the changing character of recent influenza epidemics in Britain. In 1941–42 Hirst serum tests were made on 52 sporadic cases of clinical influenza and atypical pneumonia, but the results were entirely negative. A few garglings were also examined from military units, but ferrets could not be infected with this material and it was concluded that no influenza-virus infection of known type was current during this period. During 1942–43 an investigation of acute respiratory infections was carried out in two divisions in which cases were officially notified (Stansfeld & Stuart-Harris, 1943). In division B, the cases were divided into febrile and afebrile, but in division A they included all cases of colds, sore throat, influenza, bronchitis and pneumonia, whether febrile or not. The incidence of respiratory infection was low in both divisions but sporadic cases of influenza occurred in some regiments.

During the entire period in division A, and from January to March in division B, tests were made on garglings and on the sera collected from cases of upper respiratory-tract infections. The entire series of serological tests in 1942–43 was made with reconstituted dried allantoic fluid and Hirst's (1942) new technique was used. Of 114 sera tested against virus B, 52 showed no change, a decrease in titre, or an increase not exceeding 1½-fold; 12 showed a 2- to 3-fold increase, and 50 showed an increase of 4-fold or greater. The sera of all 52 in the first category, of 11 in the second, and of 15 in the third were also tested against virus A. Only 2 pairs of sera showed differences in titre with virus A in excess of 3 times; the majority showed no change. These results were interpreted as indicating infection with influenza-virus B in 50 patients. It was noted that the rise of B-antibodies in individual patients was correlated with the initial titre of the serum, so that patients with low initial levels of antibody more often showed increases than those with high initial levels.

Towards the end of the normal influenza season, and after it, there was a number of small outbreaks apparently due to influenza A. Two or three cases of influenza at the end of March showed increase of antibody to influenza A but not to B. Further localized outbreaks in March and again in May and in June 1943 were also found to produce significant rises in titre against virus A.

Attempts were made to recover, by means of egg-inoculation, strains of influenza virus from garglings of the patients who did, or did not, show increases in B-antibody. The

garglings were filtered and inoculated amniotically into 12-day chick embryos, but the results from 21 patients were negative. Garglings from 26 patients were inoculated intranasally into ferrets between January and April 1943 and the results, at first, were entirely negative. However, in 8 ferrets inoculated with the original material there was a 4- to 16-fold increase in the blood level of antibody against B-virus. The specificity of the antibody in the ferret sera was confirmed in 5 instances by neutralization tests in mice. It was possible to make a comparison between sera from human cases and sera from ferrets inoculated with the corresponding garglings in 21 instances. In 7 cases, both human and ferret sera were negative: in 6, both were positive; in the remaining 8, the human sera were positive, and the ferret sample was negative.

Recent serological tests (Lush, Stuart-Harris & Andrewes, 1941) have shown that a considerable proportion of cases of influenza towards the end of 1939 was due to B-virus. This was the first record of this strain in the British Isles. In 1940–41 there were mild outbreaks, some of which were due to A-virus. During the early months of 1942 there were no widespread epidemics and no evidence of infection with virus A or B was obtained. Towards the end of the year, however, and again in January and February 1943, a mild increase of acute respiratory infection was associated with serological evidence of influenza-virus B infection. Clinically the illnesses were typical influenza, but other respiratory infections which did not evoke the serological evidence for known influenza viruses were also occurring at the same time. Subsequently, from March to June, the type of infection changed again and minor outbreaks of influenza A infection occurred in the country. Later on, in November 1943, influenza appeared in epidemic form and the virus responsible was of the A type. This course of events bears some similarity to the Argentine records for the years 1940–42 (Taylor, Parodi, Fernandez & Chialvo, 1942).

Finally, the authors discuss the value and limitations of the Hirst technique in detecting virus-B infections. Provided that the factors causing variation in the inhibition titres of any particular serum are fully recognized and suitable precautions are taken (Stuart-Harris, 1943), the test is exceedingly useful. The authors confirm the findings of Taylor *et al.* (1942) on the value of instilling garglings into ferrets and subsequently examining the animal's serum by the Hirst technique. Serial passages through ferrets were also found to induce a rise in antibody, although no overt symptoms of infection could be recognized.

REFERENCES

Hirst, G. K. (1942) *J. exp. Med.* **75**, 49

Lush, D., Stuart-Harris, C. H. & Andrewes, C. H. (1941) *Brit. J. exp. Path.* **22**, 302

[1] Stansfeld, J. M. & Stuart-Harris, C. H. (1943) *Lancet*, **2**, 789

Stuart-Harris, C. H. (1943) *Brit. J. exp. Path.* **24**, 33

Taylor, R. M., Parodi, A. S., Fernandez, R. B. & Chialvo, R. J. (1942) *Revist. Inst. bact. C. G. Malbran*, **11**, 44

[1] [*see BMB* **558**]

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INFLUENZA "A": AN ACCOUNT OF A MINOR EPIDEMIC

by T. H. Donnelly, H. P. Hughes, D. Robertson & E. Philipp, *British Medical Journal*, **1**, 42–43, 8/1/44

This account, by medical officers of the Royal Air Force and Royal Australian Air Force, gives clinical and pathological details of an outbreak involving 68 cases at an airfield. A Canadian airman went sick with symptoms of influenza on March 30, 1943; thereafter single cases occurred on April 3, 14 and 16. Ten cases were reported on April 21 and it then appeared obvious that an epidemic was developing. On April 23 some of the 30 patients then in sick quarters were examined by: (i) chest radiography; (ii) differential blood count; (iii) throat swabs; (iv) blood culture; (v) sputum examination; (vi) titration of antibodies in the blood against influenza viruses A and B. In most cases headache was severe, there was slight soreness of the throat, nasal catarrh, nausea and low backache. Temperatures varied between 102° and 103° F. [about 39°–39·5° C.]. There was little of diagnostic value in the blood counts, throat-swab examinations, or blood cultures. Sputum examinations disclosed one case of pulmonary tuberculosis, but otherwise were negative. Only one case showed radiologically congestion and some consolidation of the right base. Titrations of antibodies in 6 cases against influenza viruses A and B by the Hirst technique showed no significant change in antibody level against virus B, whereas 4 showed a good rise (6- to 16-fold) against virus A. It is suggested that some of the cases which showed no increase of A-antibodies may already have had good A-antibodies in the serum and the infection may have failed to cause a further rise (Andrewes, 1942). The authors discuss the methods of treatment of such an epidemic and consider that sulphapyridine was useful in preventing subsequent pneumococcal or streptococcal infections. They also emphasize the importance of bearing influenza in mind when patients develop unusual symptoms, and they point out that failure to do so may mean the introduction of influenzal infection into hospitals.

REFERENCE

[1] Andrewes, C. H. (1942) *Proc. roy. Soc. Med.* **36**, 1

[1] [*see BMB* **65**]

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THE INFLUENZA "A" OUTBREAK OF OCTOBER–DECEMBER, 1943

by C. H. Andrewes & R. E. Glover, *Lancet*, **2**, 104–105, 22/7/44

During the last three months of 1943 there occurred in Britain the most widespread outbreak of influenza since 1937. A small number of cases of influenza in the early months of the year was found to be due mainly to influenza-virus B; but in April localized and small outbreaks caused by virus A began to be observed. Sera from patients involved in the main outbreak were examined by workers at the National Institute for Medical Research in London and subjected to the Hirst test—the titration in sera of the property of inhibiting the agglutination of fowl erythrocytes by influenza virus. Serum samples were taken from clinically typical patients early in the disease and again 10–14 days later. Using a 4-fold rise in titre as significant of infection, 72% of the 60 sera examined were positive for virus A, while if a 2-fold rise in titre was taken as significant 82% had been infected with virus A. No rises in titre against influenza-virus B were found.

In addition to the Hirst tests, unfiltered garglings from 24 patients were inoculated into ferrets and typical symptoms were produced with 14 of the specimens. Material from most of the positive or doubtful ferrets was passed intranasally to more ferrets by inoculating ground suspensions of lung and turbinates, or nasal washings. The latter were obtained by allowing the anæsthetized ferret to breathe for 30 seconds with its nose intermittently under the surface of 5 $cm.^3$ of broth in a Petri dish (Burnet & Bull, 1943). This gave material for passage while allowing the animal to be kept for serological study. In contrast to results obtained by the authors in the previous few years, garglings inoculated into ferrets either produced definite symptoms and virus was readily transmitted in series, or the tests were unequivocally negative.

Adaptation to mice was attempted in 17 instances from the ferret material of 9 of the strains. After 2–6 ferret passings, pooled lung- and turbinate-suspensions were inoculated intranasally into mice without filtration, and at least 4 serial passages were carried out. Only 2 of the strains could be adapted in this way, despite various expedients to improve on these results. Adaptation to chick embryo was attempted with filtrates of 4 garglings which were inoculated amniotically into 10-day chick embryos. Virus was then sought for by Hirst tests on amniotic and allantoic fluid, but without success.

If it can be assumed that the material available for the present study was representative of that to be found in the country generally, it seems that the October–December 1943 outbreak was due to influenza-virus A. Influenza of a similar type occurred in the U.S.A. in the autumn of 1943, and it is noted that, there also, the epidemic was preceded in the spring by small outbreaks due to A-virus. The ease with which ferrets could be infected supports Andrewes' (1942) contention that there may be a correlation between the extent of epidemic spread in man and the ease with which laboratory animals can be infected. This theory, however, was not

borne out so far as mice were concerned. The authors draw attention to their lack of success in infecting chick embryos with human material and suggest that the breed or diet of the fowls providing the eggs may be important.

REFERENCES

[1] Andrewes, C. H. (1942) *Proc. roy. Soc. Med.* **36**, 1
Burnet, F. M. & Bull, D. R. (1943) *Aust. J. exp. Biol. med. Sci.* **21**, 55

[1] [*see BMB* **65**]

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INFLUENZA VIRUS AS A LABORATORY CONTAMINANT

by C. H. Andrewes, R. E. Glover, F. Himmelweit & W. Smith, *British Journal of Experimental Pathology*, **25**, 130–134, August 1944

It is not generally recognized that viruses can act as contaminants in much the same way as bacteria. Recently-developed techniques, such as the blind passage of material from one animal to another, increase the chances of such contamination: a trace of virus accidentally introduced during passage may make its appearance after one or two further passages. Workers in the National Institute for Medical Research (Hampstead) and at *St. Mary's* Hospital, London, were led to investigate this possibility by two incidents which occurred during the course of their own work and the evidence they present is highly suggestive.

The W.S. strain of influenza virus, originally isolated by the Hampstead workers in 1933, is antigenically distinct from the vast majority of A strains, and in addition it has other biological peculiarities. Since 1933, no W.S.-like strain has been shown to be the agent responsible for an outbreak of influenza. Yet W.S. virus has made its appearance in various laboratories under the following circumstances: (i) Worker A in country Z recovered an influenza A strain serologically like W.S. from an outbreak. The strain was, at the time, the only one known to have been isolated by this worker. The Hampstead laboratory had previously sent W.S. virus to A. (ii) Worker B in country Z also recovered a W.S.-like strain from an outbreak and he too had previously been sent the W.S. strain. (iii) Worker C in country X recovered a single strain of the W.S. type from an outbreak. None of the numerous strains recovered by other workers from the same outbreak was like the W.S. strain. W.S. virus had previously been sent to C. (iv) Worker D in country Y recovered W.S. virus from normal mice, although other workers have failed repeatedly to do so. W.S. virus had been previously sent to D. (v) Worker E in country W recovered the only strain known to have been found by him from an influenza patient. This strain was identical with the W.S. strain previously supplied to him. (vi) Worker F in country V recovered several strains of influenza from normal carriers although other workers have failed to do so. One of these strains proved to be exactly like the W.S. virus previously sent to him.

In addition to this evidence, certain happenings in the authors' own laboratories strengthened their suspicions. W.S. virus, for example, was recovered from vaccinia virus which was being used to infect mice; a different strain of virus sent to a worker G was found to be contaminated with W.S. virus after a few mouse-passages; another worker recovered the W.S. strain after a few passages in mice from material from a sufferer from the common cold. At the time of all of these incidents, W.S. virus was under study in the laboratories concerned. Three further accidents of a similar nature also occurred in the same laboratories at a time when the influenza-virus strain PR8 was under study. In these three cases the contaminating organism was the PR8 strain.

In view of the possible transference of influenza infection from mouse to mouse within a cage, or from cage to cage, experiments were undertaken to determine whether this was a possible explanation of virus contamination. An attempt was made to confirm the experiments of Eaton (1940) who had shown that influenza could spread readily from mice infected with heavy doses of virus. The early experiments seemed to confirm the American work, but later it became much more difficult to demonstrate spread of the virus among mice. In any event, infection of normal stock is unlikely to explain the contamination since laboratory infection does not spread from one mouse jar to another.

While it is possible that some of the 14 instances described in this paper may be explained on other grounds than as contamination, the evidence presented is very suggestive. It seems that influenza virus may appear as a laboratory contaminant under conditions not yet understood. The important implications of this work are obvious, and the authors make the useful suggestion that, where a strain of virus is recovered under unusual circumstances, it should be stated whether stock strains of the particular virus have or have not been recently under study in the laboratory concerned.

REFERENCE

Eaton, M. D. (1940) *J. Bact.* **39**, 229

Poliomyelitis : Vaccinia

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POLIOMYELITIS : EXPERIMENTAL WORK IN EGYPT

by C. E. van Rooyen & A. D. Morgan, *Edinburgh Medical Journal*, **50**, 705–720, December 1943

During the years 1941 and 1942, 106 cases of poliomyelitis occurred among troops of the Middle East Forces, and of these 33 died. The present paper by two British Army pathologists gives clinical and histological details of 7 of the fatal cases together with an account of the methods adopted in demonstrating the infecting virus.

The lesions encountered were in the main similar to those found in England except that they were more extensive and severe. While the severity varied at different levels, pathological changes were usually found throughout the length of the cord. The anterior horn cells showed swelling, loss of outline, chromatolysis and karyolysis, only an occasional cell at the periphery of the grey matter escaping. Infiltration with polymorphs and lymphocytes (the former predominating in fulminating lesions) was observed throughout cross sections of the grey matter. It was noted that while almost all cases died of medullary failure extensive lesions were usually found as high as the pons and mid-brain and even in cases showing nothing but bulbar paralysis slight lesions could be found as low as the lumbar enlargement.

The experimental animals employed in the study were the Sudanese monkey, *Cercopithecus aethiops*, and the Abyssinian baboon, *Papio hamadryas*. The latter species was found to be highly susceptible to the disease. Segments of spinal cord from fatal cases of poliomyelitis were emulsified in glycerol saline of *p*H 7·2, allowed to stand for 3 days and then tested by aerobic and anaerobic culture for viable bacteria. Thereafter a 1 : 20 emulsion of the tissue was prepared, centrifuged at 3000 revolutions per minute for 30 minutes, and 0·25 cm.^{3} of the supernatant fluid was inoculated intracerebrally. By this technique poliomyelitis virus was demonstrated in 6 of the 7 fatal cases, the animals usually dying in from 5–8 days with the typical clinical and histological features of poliomyelitis. In view of the ease with which the disease could be induced in *C. aethiops* an attempt was made to test the prophylactic and curative effect of sodium sulphadiazine in experimental infections. The drug had no appreciable effect on either of the two monkeys used.

In addition to known cases of poliomyelitis, a small number of neurological cases in which the clinical signs were suggestive either of choriomeningitis, encephalitis, or brachial neuritis were also investigated. Tests were made at the Rockefeller Institute for Medical Research to ascertain whether the sera of these patients contained neutralizing antibodies against a number of known virus infections. The sera from 9 out of 11 of these cases gave positive protection when subjected to the Lansing-strain poliomyelitis-virus intracerebral mouse-protection test. A control experiment with sera from normal

troops would have enhanced the value of these observations. The authors conclude this paper by noting the striking difference in the incidence of poliomyelitis among the troops compared with that among the civilian Egyptian population. According to available evidence only 11 cases of poliomyelitis occurred in Egypt during the years 1938 and 1939.

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POLIOMYELITIS IN BRITISH AND AMERICAN TROOPS IN THE MIDDLE EAST: THE ISOLATION OF VIRUS FROM HUMAN FAECES

by J. R. Paul, W. P. Havens & C. E. van Rooyen, *British Medical Journal*, **1**, 841–843, 24/6/44

The present report, by pathologists of the American and British Forces, is mainly concerned with attempts to isolate virus from the stools of typical and borderline cases of poliomyelitis which occurred among troops stationed in Libya, Egypt and Palestine. They sought to establish the local value of the procedure in diagnosis, and to find out whether adult sporadic cases harbour the virus in the intestinal tract in the same way as juvenile cases do in areas where the disease is epidemic. Not much help was obtained in explaining the extreme severity of the type of poliomyelitis met with by studying the published information about the disease in the countries concerned. Only 13 cases were officially recorded for the years 1938 and 1939 in Egypt, but unofficial records suggest that poliomyelitis was not uncommon especially among children under 5 years of age. Records also suggest that it is not rare in Palestine, especially among Jewish children.

Among British troops 74 cases occurred in 1941, with 19 deaths, while in 1942 there were 32 cases with 14 deaths. The rate among American troops in the Middle East during the first 10 months of 1943 was more than 10 times that recorded in the U.S.A. for a similar period. Attempts to obtain an epidemiological link between 10 of the military cases and cases occurring among the civilian population were unsuccessful; the military cases were ubiquitous in their place of origin and no two patients seemed to have been in contact.

The advantages of testing stools rather than nasopharyngeal washings in poliomyelitis rest on the fact that in juvenile cases the virus remains in the intestinal tract for some 20 days whereas it is present for only 2 to 4 days in the nasopharynx. Information about the presence of virus in the stools of adults, however, is incomplete. Stool specimens from 35 patients and contacts were tested: 17 of these were American and 18 British. Five types of case were studied: (i) 15 typical cases of poliomyelitis, 10 of which were fatal; (ii) 5 cases diagnosed as polio-encephalitis; (iii) 6 cases diagnosed as acute benign lymphocytic meningitis; (iv) 6 cases of localised neuritis; (v) 3 poliomyelitis contacts.

Methods employed in demonstrating the virus were to keep stool specimens or enema material refrigerated not more than two weeks before preparing a 10% suspension in sterile distilled water and allowing it to settle at room temperature. The supernatant fluid was then divided and part was instilled in 3 cm.^{3} amounts into the nostrils of a monkey on three or four successive days. The second part of the fluid was immediately centrifuged and 15% ether was added to the supernatant and refrigerated for 48–72 hours before intraperitoneal inoculation in 15–20 cm.^{3} quantities into the same monkey. Five different species of monkey were used in these experiments: (i) grivet monkeys, *Cercopithecus griseoviridis*; (ii) Central African vervet monkeys, *Cercopithecus aethiops centralis*; (iii) small immature Abyssinian baboons, *Papio hamadryas*; (iv) Hussar monkeys, *Erythrocebus patas*; (v) the bonnet monkey, *Macacus radiata*. The first four of these species were shown to be susceptible to poliomyelitis virus but the fifth was not adequately tested. In all, 44 monkeys were used, 6 of them twice.

Of the 15 poliomyelitis cases tested, the stools in 9 were shown to contain virus. Positive results were based on clinical signs, such as weakness and paralysis, and on histopathological evidence from the medulla and cord of infected animals. All patients from whom virus was obtained died. The results suggested that the amount of virus present in the intestinal tract was greater in the more severe cases of poliomyelitis than in the milder cases. It is noted, however, that the faeces from the fatal cases were collected earlier in the course of the disease (from 3–10 days) than in the other cases. All tests for virus in the stools from cases other than typical poliomyelitis were negative.

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ULTRAMICROSCOPICAL OBSERVATIONS ON THE MORPHOLOGY AND DEVELOPMENT OF VACCINIA VIRUS *IN VITRO*

by K. B. E. Merling, *British Journal of Experimental Pathology*, **24**, 240–245, December 1943

The morphological appearances of living vaccinia virus in the corneal cells of rabbits have already been described by the author, who is a research fellow at the Bland-Sutton Institute of Pathology in London. In the present paper he reports a study of the life-cycle of vaccinia virus and describes survival of the virus *in vitro* in its various forms. The eyes of rabbits were scarified with the virus and, after keratitis had developed, thin tangential sections were cut with a sharp knife, while from the deeper layers scrapings were obtained with the blade of a knife. Control preparations from uninoculated eyes were also made. The material was placed in a drop of tyrode solution on a thin slide under a cover slip, which was sealed with paraffin wax. Observations were carried out at room temperature or on a warm stage at 37° C. using optical systems giving a high degree of resolution at the highest magnifications with dark-ground illumination. A number of photomicrographs of the more important findings are printed in the text and study of these is essential for appreciation of this article. It is not possible to describe these fully here, but the three forms of vaccinia virus which represent its life-cycle can be readily seen. Elementary bodies, discs and spheres together with secondary or daughter-cysts are illustrated. In some of the photographs of chain formations gaps occur and these are occupied by discs which are not recorded owing to their low brightness. No connecting filaments, as described by Paschen (1906) and Prowazek (1907) were visible. One photomicrograph shows the elementary bodies at a magnification of 2900, and clearly demonstrates their oblong shape. The resolution achieved in this photograph is remarkable because it is beyond the predicted possibilities of the Abbe and other image-formation theories of the microscope. The raising of the limit of resolution and of magnification was achieved by reducing the intensity of the light-source by polarized discs to such a degree as to produce coincidence between the minimum of photographic registration and the physiological thresholds of perception and differentiation of two light intensities.

Other figures illustrate large numbers of small colonies of virus which appear to have a very thin membrane holding their contents together. The small cysts are not round, as is the rule with those formed from cell extensions, but appear in a variety of shapes whose most impressive feature is change. On examining these cysts a week later, they appear to have grown to large dimensions, measuring 20 × 30 μ across their two diameters. The cysts may then stretch and divide into two or more, and following the division they may move across the field. Continuous small cysts are formed, grow and divide in seemingly endless repetition until about six weeks after the material is taken from the animal, when the process slowly comes to an end. It was noted that the formation of new colonies could take place after the complete disappearance of host-cells and of their broken fragments: evidently the metabolic products present in the liquid were stable and sufficient for the development of new colonies. Gradually, however, the cysts cease to be formed and the number of free-moving bodies also decreases. The end of the process of development of the virus in these specimens was immobilization with loss of brightness of the virus bodies. Control specimens of normal cornea kept for the same time showed cells in varying degrees of disintegration, but were negative for virus bodies.

REFERENCES

Paschen, E. (1906) *Münch. med. Wschr.* **53**, 2391
Prowazek, S. von (1907) *Arch. Protistenk.* **10**, 336

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INCLUSION BODIES IN ASSOCIATION WITH TYPHUS RICKETTSIAE

by A. M. Begg, F. Fulton & M. van den Ende, *Journal of Pathology and Bacteriology*, **56**, 109–114, January 1944

When typhus rickettsiae have become well adapted to a particular animal host, the organisms usually appear in the form of typical minute bacillary and coccobacillary forms; in smears from the tissues or exudates of infected animals the rickettsiae are to be found in large numbers in the cytoplasm of mononuclear cells as well as extracellularly. In this study by Begg and his colleagues at the National Institute for Medical Research, the appearances seen in the lungs of animals during the adaptation of rickettsiae to a new host-species have been followed.

. Most of the observations were made on lung impression-preparations stained by Macchiavello's method. When the mouse-lung-adapted strain of the epidemic type (Breinl strain) was passed into the lungs of rabbits, no bacillary forms were found at first, but only small intracytoplasmic homogeneous inclusion bodies ; with repeated passage, small granular inclusions, in some cases obvious small clusters of rickettsiae (morulae), began to appear in some cells ; and in later animals, when the strain had become adapted to the rabbit and regularly produced consolidation of the lungs, rickettsiae were also found diffusely scattered in the cytoplasm of cells as well as extracellularly.

When mouse-lung-adapted rickettsiae of the murine type were passed into the lungs of rats homogeneous inclusion bodies were not found, but all stages described in the rabbit, from the morula onwards, were seen. Homogeneous inclusion bodies were not found in the lungs of mice at any stage of the infection with mouse-lung-adapted strains. However, while new strains from infected guinea-pig brain were being adapted to mouse lung, these inclusion bodies were easily found. As during adaptation to rabbit lung, the homogeneous inclusion bodies began to decline in number with the appearance of morulae and bacillary forms. The various forms described in lung preparations are beautifully illustrated by photomicrographs in colour. The authors believe that the homogeneous inclusion bodies occur only during adaptation of a strain of rickettsiæ to a new host, and it is suggested that the morulae are more common when the invaded cell is somewhat resistant. While it appears that the homogeneous inclusions may be the most immature forms of morula, the authors consider that the evidence provided by ultra-violet photomicrography is rather against this view.

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RICKETTSIAL AGGLUTINATION STUDIES IN TYPHUS FEVER

by C. H. Stuart-Harris, G. K. C. Rettie & J. O. Oliver, *Lancet*, **2**, 537–538, 30/10/43

This work, by a group of army pathologists engaged in typhus research, affords further evidence of the value of agglutination tests with suspensions of typhus rickettsiae in this disease. The suspensions were prepared from infected mouse lungs, 3 strains of rickettsiae being employed. These were the Wilmington murine strain, the classical Breinl strain of epidemic typhus, and a strain isolated in 1942 by van Rooyen from a case of louse-borne typhus in Cairo.

Sera were examined from nine cases of louse-borne typhus fever which had occurred in the West of Ireland in 1942, as recorded by McConn (1943). The sera were obtained 2 to 5 months after subsidence of the fever. Significantly high titres of agglutinins to *Proteus* OX19 were present in the sera of 5 of the 9 cases and high OX2 titres in 2 of these and 1 other. Rickettsial agglutinins to the Cairo strain were present in 7 sera and 2 of these showed no agglutinins to the Breinl or murine rickettsiae. Five of the sera reacted with all 3 rickettsial strains ; 4 of these showed a higher titre with the epidemic than with the murine organism, the fifth having similar titres with all 3 strains.

The authors note that, while sera from human cases of typhus fever usually showed both rickettsial and OX19 agglutinins, experimental infection of guinea-pigs and rabbits produced sera which agglutinated rickettsiae but had either no, or only a weak, titre of agglutinins to OX19. A comparison of the results with sera from cases of typhus fever and from experimentally infected rabbits and guinea-pigs showed that, with human and rabbit sera, the titre of agglutinins for the infecting type of rickettsiae—epidemic or murine—was higher than for the other type, but that some cross-agglutination occurred in each case ; with guinea-pig sera, however, the agglutinins tended to be specific for the infecting organisms. Cross-absorption of rabbit immune-sera with suspensions of rickettsiae of the 2 types supported other evidence that the 2 species of rickettsiae—*R. prowazeki* and *R. mooseri*—possess specific as well as a common antigen.

These findings together with the work of van Rooyen & Bearcroft (1943) and others suggest that rickettsial agglutination tests with the sera of typhus patients are more likely to indicate the nature of the infecting strain of rickettsiae than are Weil-Felix tests. The data available indicate that the cases of typhus in Ireland were due to *R. prowazeki*. The authors comment on the absence of a typhus epidemic from this part of Ireland since 1905, and speculate on the mode of survival of the organism in inter-epidemic times.

REFERENCES

McConn, C. F. (1943) *Lancet*, **2**, 525

[1] van Rooyen, C. E. & Bearcroft, W. G. C. (1943) *Edinb. med. J.* **50**, 257

[1] [*see BMB* **151**]

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ENDEMIC TYPHUS FEVER IN DIEGO SUAREZ, MADAGASCAR

by C. G. Baker, G. T. L. Archer & G. B. Mitchell-Heggs, *British Medical Journal*, **2**, 506–508, 23/10/43

According to the local public health report for Madagascar in 1940, it was believed that the typhus group of diseases did not occur on the island. Major Baker and his colleagues in the Royal Army Medical Corps, however, have recorded in this paper a series of 10 cases which, in their clinical aspects, were typical typhus infections of mild or moderate degree of severity. The cases occurred among Service units in 1942. The clinical features of the illness are discussed and symptoms are tabulated. All 10 patients showed agglutinins in their serum to *Proteus* OX19 in dilutions of 1 : 200 or more, and in several of the patients in whom repeated examinations were made the agglutinin curve showed the typical rise and fall. Titres for OXK were negligible, and in only 1 patient was the titre for OX2 as high as for OX19. The authors were unable to decide which insect vector had been concerned in the transmission of infection. None of the patients was louse-infested, but all had been bitten by insects recently; fleas from rats found in the native huts, which were at one time used by a few of the men for a short period, and fleas from rats living in an ammunition dump, which had been guarded in turn by most of the patients, were possible vectors. *Xenopsylla cheopsis* and *X. braziliensis* were found in rats caught in the neighbourhood, and ticks infested almost every dog in the district. Lack of facilities prevented laboratory investigation to determine the insect vector and possible animal reservoir. The authors suggest that the results of the Weil-Felix tests resemble those which occur in the " OX19 group " of typhus cases in India.

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VACCINATION AGAINST TYPHUS FEVER

by J. B. Penfold, *British Medical Journal*, **1**, 114, 22/1/44

Agglutinins to *Proteus* OX19, OX2 and OXK suspensions are usually absent, or present in only low dilutions, in the blood serum of the normal English population. Felix (1944) has recorded the agglutinin titres for these suspensions in the blood serum of persons who had been inoculated with vaccines of typhus rickettsiae.

Dr. Penfold, a member of the Emergency Medical Service, has recorded similar findings in 23 persons who had been inoculated with a typhus vaccine prepared in Toronto from a louse-borne strain of *Rickettsia prowazeki* grown in the yolk-sac of chick embryos. Each person received 0·25 cm.^{3}, 0·5 cm.^{3}, and 1·0 cm.^{3} of vaccine subcutaneously at weekly intervals and 3 months later a fourth dose of 1·0 cm.^{3}

Samples of blood were obtained for test (i) before the first injection, (ii) a fortnight after the third injection, (iii) before and (iv) a fortnight after the fourth injection. Sera were tested in dilutions from 1 : 5 to 1 : 640 with suspensions of *Proteus* OX19, OX2 and OXK. The tests were kept 4 hours at 56° C. and overnight at room-temperature before the results were read.

In the majority of cases there was an increase in the titre of agglutinins to all three suspensions after the first three injections. After the fourth injection the titres for OX19 and OX2 again rose, while the OXK titres were not appreciably altered. Even after the fourth injection, however, the highest titre for OX19 was 1 : 160 and for OX2, 1 : 80. If an increase of more than 100 % in agglutinin titre for OX19 is regarded as significant, 14 of the 23 (61 %) showed it after the first three injections and 17 (74 %) after the fourth injection of the rickettsial vaccine. Reactions to the injections of vaccine were usually slight.

REFERENCE

[1] Felix, A. (1944) *Trans. roy. Soc. trop. Med. Hyg.* **37**, 321

[1] [*see BMB* **572**]

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NOTES ON TYPHUS FEVER IN THE MIDDLE EAST

by W. Brockbank & S. R. F. Whittaker, *Lancet*, **1**, 150–151, 29/1/44

The authors record clinical observations and laboratory findings in 10 sporadic cases of typhus fever admitted to a military hospital in the Middle East over a period of three months. All the patients were British soldiers and most of them had duties which brought them into close contact with civilian labour. Evidence of lice was found only in 1 case. Of the 10 cases, 8 were admitted to general wards where they remained until the rash appeared on the fifth or sixth day of illness. The authors comment on the difficulty of early diagnosis in the absence of an epidemic. In 2 cases, appearance of the rash was delayed until the 10th day and in these 2 the Weil-Felix test was of considerable help. In all cases the titre of agglutinins for OX19 was 1 : 120 or higher at some stage of the illness but, with the exception of the 2 cases in which the rash appeared late, the results were not significant until the clinical diagnosis was fairly certain. In 3 cases, the titre of agglutinins was higher for OX2 than for OX19, in 6 it was higher for OX19 than for OX2, while in 1 patient, who died on the 9th day of illness, the titre was the same (1 : 120) for both suspensions. In 6 cases specific rickettsial agglutination tests were made by Major van Rooyen at the Central Pathological Laboratory, Middle East Forces, and the diagnosis of louse-borne or epidemic typhus was confirmed. No case of cross-infection occurred, although several patients had been in general wards before delousing was carried out.

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DRY BLOOD TEST FOR TYPHUS FEVER : Preliminary Report

by P. N. Bardhan, N. Tyagi & K. Boutros, *British Medical Journal*, **1**, 253–254, 19/2/44

This preliminary report by medical officers of the Army Medical Corps from a field laboratory with the Middle East Force deals with a slide agglutination test; such tests have been used in the German Army on their Eastern Front where typhus fever has been a considerable risk during the war. The authors used a simple technique: two drops of blood obtained by pricking the ear or a finger were placed on a clean glass slide and allowed to dry. A drop of concentrated suspension of *Proteus* OX19 was placed on one blood drop and OX2 suspension on the other. The slide was allowed to stand for one minute and then the blood was mixed with the suspensions by rocking the slide. The slide was set aside but rocked occasionally; the results were read after 5–10 minutes with the naked eye and confirmed with the hand lens if necessary.

Blood specimens from 640 Egyptian labourers in various camps under military control were examined. Thirteen specimens gave positive results with OX19, and 2 of these were positive to OX2 as well. These 13 were further examined by a complete Weil-Felix test in agglutination tubes. Of the 13 sera, 7 gave positive results with OX19 suspension, and 2 of these 7 agglutinated OX2 to a dilution of 1 : 50. These last 2 were from the same persons whose blood had given the positive results with OX2 on the slide. Weil-Felix tests were repeated at 4–7 day intervals on the 7 patients giving positive results. All of them gave a positive reaction to OX19 in serum dilutions of 1 in 250 or more in at least one of the tests. The history of these men showed that 3 were typhus cases, 2 were recently convalescent from typhus fever, while the remaining 2 could not be traced.

These observations suggested that, while all the typhus fever cases gave a positive slide test, a positive result with the slide test did not necessarily indicate a typhus infection. Thirty-three of the bloods which had given a negative slide test were tested by the full Weil-Felix technique; 25 of these were negative at 1 : 25 serum dilution, while none of the remaining 8 were positive in a serum dilution higher than 1 : 50 with either OX19 or OX2 suspension. The authors suggest that the slide test with dried blood might serve as a useful preliminary diagnostic method and that a complete Weil-Felix test need not be done unless the slide test is positive; they recommend that the slide method be tried out on a more extensive scale.

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TECHNIQUE AND INTERPRETATION OF THE WEIL-FELIX TEST IN TYPHUS FEVER

by A. Felix, *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **37**, 321–341, March 1944

As suspensions of rickettsiae are not yet generally available for routine use in the diagnosis of the typhus group of fevers, the Weil-Felix reaction is likely to remain the standard test in many laboratories for some time. This article, written by one of the originators of the test at present working for the Emergency Public Health Laboratory Service, should prove helpful to pathologists required to assist in the diagnosis of suspected cases of typhus fever. The greater part of the paper is concerned with louse-borne typhus. Curves of agglutinin response to *Proteus* OX19 and OX2 in the sera of typhus patients studied in Poland and Turkey by the author during the last war are reproduced. The majority of cases of louse-borne typhus develop agglutinins for OX19 about the 4th or 5th day of illness, and the maximum titres reached are high. Moderately severe cases tend to show this type of response. In the most severe cases, however, a positive reaction tends to appear later, about the 6th or 7th day and the maximum titre reached is usually low (below 1 : 5000). The mildest cases may have either very low or high titres.

It is pointed out that, in sera from persons in countries free from typhus, agglutinins for *Proteus* OX19 and OX2 are only occasionally present, and then usually of titres below 1 : 100, which is frequently taken as the limit of normal agglutinins. In countries where typhus is endemic, agglutinins of much higher titre may be present in the sera of persons who have suffered from clinical or inapparent infection many months previously. Non-specific stimulation of OX agglutinins by other febrile infections (anamnestic rise) does not occur. Felix's experience agrees with that of Penfold (1944) in showing that OX19 agglutinins are produced irregularly, and only to low titre, following vaccination of healthy subjects with egg-yolk rickettsial vaccine; such agglutinins do not affect the diagnostic value of the Weil-Felix test, as a rising curve of OX agglutinins is diagnostic of typhus in the inoculated as well as in the uninoculated. Repeated tests on the serum are of especial importance in early diagnosis. When only the results of a single test are available, complete agglutination of a standard suspension in a serum dilution of 1 : 100 may be considered significant in an uninoculated person who is not a native of an endemic area. If the patient has been inoculated with rickettsial vaccine 2 or 3 months before his illness, complete agglutination at a titre of 1 : 200 or more may be regarded as strongly suggestive. Lower titres than these in the early stage of illness should not be ignored.

The author recommends keeping the tests at 37° C. for 2 hours and overnight at room-temperature before the final reading of results; the simple slide tests (see Bardhan,

Tyagi & Boutros, 1944) may be of value under the exceptional conditions which call for their use.

In murine typhus, agglutinins for OX19 generally appear in the patient's serum during the febrile period and have the same significance as in louse-borne typhus; these two varieties of the disease can be differentiated serologically only by tests with rickettsial suspensions (van Rooyen & Bearcroft, 1943; Stuart-Harris, Rettie & Oliver, 1943).

In the mild tick-borne typhus fever of the countries bordering the Mediterranean (fièvre boutonneuse), irregular results are obtained in agglutination tests with *Proteus* OX antigens. Significant reactions occur late in the disease and the titres are usually lower than in louse-borne typhus; agglutinins for OX2 are usually higher than for OX19. In tick-borne typhus in India, the types of agglutinin response to OX antigens have not yet been established. In scrub typhus or tsutsugamushi of the Far East, agglutinins for *Proteus* OXK only appear in the blood. The agglutination tests with this antigen are more liable to give false results owing to instability of the suspensions, which are more liable to agglutination with normal human serum. The significant titre of agglutinins is usually taken as 1 : 200. The agglutinins, however, rarely appear before the 2nd week and may not reach a maximum until the 3rd or 4th week from the onset of illness.

REFERENCES

[1] Bardhan, P. N., Tyagi, N. & Boutros, K. (1944) *Brit. med. J.* **1**, 253

[2] Penfold, J. B. (1944) *Brit. med. J.* **1**, 114

[3] Stuart-Harris, C. H., Rettie, G. K. C. & Oliver, J. O. (1943) *Lancet*, **2**, 537

[4] van Rooyen, C. E. & Bearcroft, W. G. C. (1943) *Edinb. med. J.* **50**, 257

[1] [*see BMB* **571**] [2] [*see BMB* **569**]
[3] [*see BMB* **567**] [4] [*see BMB* **151**]

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NOTE ON THE PREPARATION OF SUSPENSIONS FOR THE WEIL-FELIX TEST

by R. F. Bridges, *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **37**, 343–344, March 1944

Lt.-Col. Bridges, of the *Medical Research Council* Standards Laboratory at Oxford, here gives details of the method used in the preparation of suspensions of the 3 *Proteus* OX strains that are employed in the Weil-Felix test. Cultures known to be suitable by preliminary tests with typhus serum are used. Roux bottles of agar are inoculated from 24-hour cultures in broth. After incubation of the Roux bottles for 24 hours, the growth is washed off with a small quantity of saline. The resulting suspension is filtered through cotton wool into screw-capped bottles and 4 volumes of 96% alcohol are added to 1 volume of suspension. The mixture is shaken thoroughly during the course of 1 hour; the supernatant alcohol is sucked off as far as possible and the remainder removed after centrifugation. The deposit of organisms is suspended in saline and shaken thoroughly to smooth out clumps; 2% buffered formol-saline (*p*H 7·6) is added to make the final concentration of formalin 0·25%. The suspension is standardized by the addition of further saline and buffered formol-saline, to a density equivalent to 4,500 million *Bact. coli* per ml. In the case of OXK suspension, the alcoholized organisms should be re-suspended in sterile distilled water in place of saline, and all further dilutions should be made with distilled water; the 2% formalin may be added in the form of buffered formol-saline as in the case of OX19 and OX2 strains.

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TYPHUS FEVER IN GREAT BRITAIN

by A. P. Agnew & W. B. Kyles, *British Medical Journal*, **2**, 10–11, 1/7/44

Although typhus fever has long since ceased to be endemic in Britain, the case recorded by Drs. Agnew and Kyles of the Emergency Medical Service in Scotland emphasizes the necessity of keeping this disease in mind in cases of unexplained pyrexia, especially among men who have recently returned from abroad.

The patient had embarked on December 20, 1943, from an area in which typhus fever was prevalent and on December 31, while still aboard ship, suffered from headache, cough and general muscular pains; his temperature was 102° F. [about 39° C.]. Bronchitis was diagnosed. From the sick-quarters on the ship he was admitted to hospital in Scotland on January 5, 1944. At this time the patient looked toxic and complained of severe headache, sore throat and cough. He had a rigor and collapsed before being put to bed, and on examination he was flushed and there were muco-pus in the nose and throat, definite bronchitis, slight general adenitis, and slight neck rigidity. His temperature was 103° F., pulse rate 80 and respirations 22 per minute. The patient's condition became slowly worse; on January 7, he was drowsy, restless and irritable; next day there was meningism, Cheyne-Stokes type of respiration and more definite evidence of bronchopneumonia. A Widal test was negative at this time. On January 9 a sparse macular rash, dusky red in colour, was present on the flanks and anterior aspects of the shoulders; the following day the Weil-Felix reaction was reported positive. After this date the patient slowly improved and by January 17, the temperature, pulse-rate and respirations were normal. The Weil-Felix test was repeated on January 15 and February 21. The highest titre of agglutinins in the patient's serum was recorded on January 15 when it was 1 : 12,800 for OX19; 1 : 400 for OX2; and 1 : 25 for OXK suspensions.

The decisive stages in reading a correct diagnosis were: (i) the recognition of typhus fever as a possible cause of the illness; (ii) the appearance of the rash; (iii) the positive Weil-Felix test. The authors consider that the patient acquired his infection before embarkation on December 20. The precautionary measures taken to prevent contact infection in the hospital are described. No secondary cases occurred and none of the other patients from the ship developed a positive Weil-Felix reaction.

Sources and Control of Hospital Infection

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DUST AS VEHICLE OF INFECTION IN CHILDREN'S WARDS

by E. D. Hoare, J. R. O'Brien & A. G. Watkins, *Lancet*, **2**, 666–667, 27/11/43

The pathogenicity of *Proteus morgani* has not been fully proven, and this report is not designed to bring evidence in this respect but rather to show the possible ways in which an organism of intestinal origin may spread throughout a ward. The paper comes from the Royal Infirmary, Cardiff, where Watkins is Physician in Children's Diseases. Hoare and O'Brien are on the staff of the Pathological Department of the Welsh National School of Medicine.

In a period of six months there were 14 infections with *Proteus morgani* in children in one ward, 10 of these appearing after admission. The infections were intestinal except in 1 case in which burns became infected. The infections could not be traced to the contamination of food during preparation or serving. Morgan's bacillus was, however, isolated from the air and the dust of the ward, from bed-linen, although not directly fouled by urine or fæces, and from flies. Experimentally, the organism was shown to survive 81 days on a blanket and up to 10 days in dust, and it could still be found on woollen garments after washing in soap and warm water. Sterilization of these garments or of blankets could be effected by exposing them for one hour to formaldehyde in a closed chamber.

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HAEMOLYTIC STREPTOCOCCI IN THE DUST OF HOSPITAL WARDS, AND THEIR RELATIONSHIP TO INFECTION: A Report to the Medical Research Council

by D. G. ff. Edward, *Journal of Hygiene*, **43**, 256–265, January 1944

The part played by dust in producing cross-infection has been the subject of increasing attention in recent years. This paper is a report to the *Medical Research Council* of

investigations on the dust in the wards of 2 contrasting types of hospital. The first investigation was made in the combined ear, nose, throat and eye ward (I) of an improvised Emergency Medical Service hospital. The second investigation was made in the children's ward (II) of another hospital. Ground-plans of these wards are reproduced in the original paper. In both cases, examinations of ward-dust were made weekly for a period of about 6 months.

The dust of ward I contained 300,000, and the dust of ward II 250,000, haemolytic streptococci per gram. Serological examination showed that about 33 % of the streptococci isolated from the dust of ward I, and about 80 % of those from ward II, were of group A. Examinations of swabs from throats and septic lesions of patients and staff showed that 27·8 % of ward II patients were infected with haemolytic streptococci either on admission or at some time during their stay in hospital. The incidence of infection acquired in hospital was more than 12·2 %. Typing of the organisms showed that the occurrence of a particular type in the dust often resulted from the presence of one or more infected persons in the ward. Conversely, it often happened that a particular type occurred in the dust before it was identified among inmates or staff of the ward. These strains may have been introduced to the ward by visitors.

There is as yet no definite evidence as to the significance of the presence of viable pathogenic bacteria in dust, and the investigations reported did not reveal a conclusive example of hospital cross-infection conveyed by dust. In the original paper the findings are reported in considerable detail.

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SOME OBSERVATIONS ON HOSPITAL DUST WITH SPECIAL REFERENCE TO LIGHT AS A HYGIENIC SAFEGUARD

by L. P. Garrod, *British Medical Journal*, **1**, 245–247, 19/2/44

The author, who is professor of bacteriology in the University of London and bacteriologist to *St. Bartholomew's* Hospital, studied the relation of dust to streptococcal cross-infections in hospital wards by determining the extent to which Group A haemolytic streptococci could be isolated from dust samples taken at different sites in the wards. It was readily shown that dust on the floors close to streptococcal cases and carriers had a high content of the organisms. Of samples from wards on the ground floor of the hospital, 72 % yielded haemolytic streptococci, whereas only 18 % of samples from the upper floor wards were positive. The lower floors were dark owing to the construction of brick walls in front of the windows, as a protection against bomb-blast, and it seemed probable that the comparatively low content of streptococci in the dust from the upper wards might be related to the free access of light.

Samples taken from different sites supported this view. Dust from, for example, well-lit window-ledges showed few haemolytic streptococci, while large numbers were usually isolated from darker situations. Streptococci in pus dried on slides survived longest in the refrigerator or a dark cupboard. They died much sooner in diffuse daylight and still more rapidly in a position exposed to sunlight, even though two layers of glass intervened.

Experiments were made with dust obtained with a vacuum-cleaner from floors known to be infected. In this dust haemolytic streptococci survived longer in the dark, and organisms of a type which had been responsible for an outbreak at the beginning of the trial were isolated from a sample kept in the dark for 195 days.

These results are further evidence that streptococcal epidemics at long intervals may be renewed by infected dust. Good natural illumination is a prime necessity for septic surgical cases. Daylight, even on a cloudy winter day in England, is lethal to bacteria, and ordinary glass is not an absolute bar to this effect.

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THE CONTROL OF DUST-BORNE STREPTOCOCCAL INFECTION IN MEASLES WARDS

by J. Wright, R. Cruickshank & W. Gunn, *British Medical Journal*, **1**, 611–614, 6/5/44

This is an account of a well-planned trial conducted by the clinical and bacteriological staff of one of the large infectious diseases hospitals of *London County Council*. The haemolytic streptococcus is the chief secondary invader in measles and the main cause of its complications, especially otitis media, mastoiditis and bronchopneumonia. The object of the trial was to find out whether oil treatment of floors and bed-clothing would reduce the numbers of haemolytic streptococci in the air during sweeping and bed-making, and decrease the incidence of cross-infections and clinical complications due to haemolytic streptococci. Two identical measles wards were chosen for the test, and conditions in respect of admissions, bed-spacing and treatment were similar throughout the 12 weeks of the trial. In the test-ward, the floors were treated with crude paraffin oil every 4 weeks, and all blankets, sheets, towels and garments were treated with white oil by the technique described by Harwood, Powney & Edwards (1944). In the control ward no such measures were taken. Air-sampling was done with the slit-sampler of Bourdillon, Lidwell & Thomas (1941).

Preliminary investigations before the test period showed that conditions in the two wards were remarkably similar. During this period only the floor in the test-ward was oiled. In both, 36–39 % of the patients were harbouring the type 6 streptococcus which, as the prevalent cross-infecting strain, was regarded as the " indicator " organism of the test ; the cross-infection rate (i.e. the percentage of patients exposed to risk who acquired type 6 haemolytic streptococci) was 53–59 % in each ward ; the middle-ear complication rates due to this type were alike ; this type prevailed in the air of both wards during bed-making.

During the test period, the mean bacterial count in the air during bed-making was 91 % less, and the mean haemolytic streptococcus count was 98 % less, in the treated than in the control ward. A similar difference during floor-sweeping was observed. The cross-infection rates due to type 6 haemolytic streptococci in the test and control wards were respectively 18·6 % and 73·3 %, and the middle-ear complication rates due to the same type were 2·8 % and 14·3 %.

The conclusion is that oiling of floors alone will not prevent dust-borne haemolytic streptococcal infection in measles wards, but that an effective degree of control is obtained when bed-clothes and linen are also treated.

The authors emphasize that the method must be further explored before it can be adopted for routine use. They also point out that measures other than those mentioned are necessary to prevent spread by direct contact by fingers, toys, etc., and by droplet spread from carriers amongst the staff and between new and convalescent patients.

REFERENCES

Bourdillon, R. B., Lidwell, O. M. & Thomas, J. C. (1941) *J. Hyg., Camb.* **41**, 197

[1] Harwood, F. C., Powney, J. & Edwards, C. W. (1944) *Brit. med. J.* **1**, 615

[1] [*see BMB* **579**]

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A NEW TECHNIQUE FOR THE APPLICATION OF DUST-LAYING OILS TO HOSPITAL BED-CLOTHES

by F. G. Harwood, J. Powney & C. W. Edwards, *British Medical Journal*, **1**, 615–616, 6/5/44

The numbers of dust-borne bacteria in hospital wards can be greatly reduced by the application of 3 % to 7 % of liquid paraffin or " technical white oil " to the bedclothes (van den Ende, Edward & Lush, 1941 ; van den Ende & Thomas, 1941 ; Thomas & van den Ende, 1941). The authors, who are on the scientific staff of the British Launderers' Research Association, were faced with the problem of devising a means of impregnating the materials with an accurate quantity of oil. It was also necessary to find a method which could be readily used in hospital laundries and would ensure the complete removal of the oil from the liquor used for soaking the clothing, so that no recovery process for the unused oil would be needed.

These problems were solved by the use of a dilute oil-in-water emulsion stabilized with cetyl pyridinium bromide. For woollen materials a positively-charged emulsion stabilized by " Fixanol C " (a cation-active agent manufactured by *Imperial Chemical Industries, Ltd.*) was used. For cotton fabrics it was necessary to apply half the required amount of oil in this way, and to complete the process by a further treatment with the remainder of the oil stabilized by an

anion-active agent, "Teepol" (manufactured by *Technical Products, Ltd.*). The liquor should be slightly alkaline, *p*H 8. By these processes all the oil used was deposited on the fabrics.

The materials must be washed and rinsed, soft water being essential. The last rinsing water is adjusted to 100° F. [about 38° C.] and the stock solution containing 20 % of technical white oil and 2 % "Fixanol" is added in requisite amount. The rotating machine runs for 10 minutes, the goods are unloaded, hydro-extracted, and dried. Cotton articles receive 2 to 3 minutes in the same solution followed by 7 to 8 minutes in a similar emulsion stabilized with 3 % "Teepol".

The oil is not greatly removed by autoclaving, or by ordinary washing, and tends to accumulate with successive oilings. Since an oil content of more than 5 % to 7 % is undesirable, later treatments may have to be adjusted to minimize this accumulation.

REFERENCES

Thomas, J. C. & van den Ende, M. (1941) *Brit. med. J.* **1,** 953

van den Ende, M., Edward, D. G. ff. & Lush, D. (1941) *Lancet,* **1,** 716

van den Ende, M. & Thomas, J. C. (1941) *Lancet,* **2,** 755

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OILED FLOORS TO CONTROL RESPIRATORY INFECTION: An Army Experiment

by P. H. R. Anderson, J. A. Buchanan & J. J. MacPartland, *British Medical Journal,* **1,** 616–617, 6/5/44

It has been shown by van den Ende, Lush & Edward (1940) and by Thomas (1941) that the number of dust-borne bacteria in the air of rooms can be diminished by treating the floors with crude paraffin oil. In order to determine whether the spread of air-borne infections could be decreased by this means, the authors, who are officers of the *Royal Army Medical Corps*, recorded over a period of 17 weeks during the winter the incidence of respiratory infections in two groups of soldiers who were living respectively in treated and untreated barracks. The units were large (1,300–1,700 men), and the soldiers in each group were similar in stage of training and living conditions. The buildings were practically identical. The medical officers agreed on a common standard for diagnosis of respiratory infection, the criteria being catarrh with obvious local signs, or a generalized infection accompanied by pyrexia.

While it is difficult to conduct a perfectly controlled experiment of this kind, the results suggest very strongly that the oiling of the floors was of value. The average weekly rate of respiratory infections in the treated unit was 7 per 1,000 men, against 38 in the control unit. An outbreak of almost epidemic proportions appeared only in the untreated unit at about the middle of the trial.

The oil, which is non-inflammable and has no unpleasant smell, is applied after thorough dry-sweeping of the floor. It dries in about 6 hours, leaving an imperceptible film. One gallon is needed to treat about 1,000 square feet [about 90 m.²] of floor-space. Subsequent applications may be made every four weeks. The oiled floor is easy to keep clean between treatments.

REFERENCES

Thomas, J. C. (1941) *Lancet,* **2,** 123

van den Ende, M., Lush, D. & Edward, D. G. ff. (1940) *Lancet,* **2,** 133

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A NOTE ON THE TRANSMISSIBILITY OF HAEMOLYTIC STREPTOCOCCAL INFECTION BY FLIES

by R. A. Shooter & P. M. Waterworth, *British Medical Journal,* **1,** 247–248, 19/2/44

Working in a hospital of the British Emergency Medical Service, the authors attempted to find whether cross-infection in hospital wards was related to the carriage of haemolytic streptococci by flies. The observations were made in two surgical wards where there were cases of streptococcal infection, and a control series was made on flies caught in the laboratory. Of 27 flies caught in the wards, 9 gave cultures of haemolytic streptococci, 3 of which belonged to serological Group A. Coagulase-positive staphylococci were also isolated. No haemolytic streptococci were isolated from the flies from the laboratory.

One of the Group A haemolytic streptococci was of type 4, a type which was responsible for a number of subsequent wound infections in this particular ward. The observations do not prove the transmission of haemolytic streptococci by flies, but suggest a possible explanation of spread of infection from case to case, or from one part of a hospital to another.

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A SCHEME FOR THE PREVENTION OF CROSS INFECTION IN CHILDREN'S WARDS

by N. M. Jacoby, *Archives of Disease in Childhood,* **19,** 26–28, March 1944

This short paper by the Registrar to the Children's Department of *Guy's* Hospital describes a new children's ward at the County Hospital, Pembury, and the precautions taken to prevent "cross-infection," i.e. infection conveyed to the child during its stay in hospital. The ward, which had accommodation for 35 children, consisted of 9 single rooms and 2 with two beds, 4 with four beds and 1 with six beds. A corridor ran the length of the ward and all but one room opened on to both the balcony and the corridor. Five of the single rooms were grouped together at one end behind swing doors, with separate bath, sluice and sterilizing rooms.

On admission each child was classified as to age and disease. The most careful isolation was given to the class most susceptible to cross-infection, i.e. infants under 18 months of age. Those with non-infectious ailments were placed in the segregated group of single rooms, and those with infections in the other single rooms. Only where infants had a similar infection to the other children, e.g. alimentary or respiratory, were they placed in a ward with others. The six-bedded room was reserved for children with tuberculosis, and children over 18 months with non-infectious diseases were grouped in the other rooms.

A high quality of nursing was considered essential to prevent spread of infection; a ratio of 5 patients to one nurse at night and 2 to one by day was desirable. Only senior nurses attended to the infants and each junior nurse had sole charge of certain older children. "Barrier nursing" was used for all infectious cases and the infants' food was prepared with full aseptic technique. Gauze masks were worn by everyone who entered the children's rooms, and few visitors were allowed.

Among the 268 children in the ward during one year 16 developed cross-infections, an incidence of 6 %. The infections were evenly scattered through the year. Two were almost certainly contracted before admission and a third was due to neglect of the rules by a cleaner.

The author concludes that these figures compare favourably with others which have been published. Further improvement would probably follow the provision of more beds, so that when necessary children could be isolated on admission till the disease was classified, and so that children of different groups need never be put together. A more impermeable type of face mask is also thought to be desirable.

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DESIGN OF DRESSING STATIONS AND CONTROL OF WOUND INFECTION

by W. Gissane, A. A. Miles & R. E. O. Williams, *British Journal of Industrial Medicine,* **1,** 90–98, April 1944

The authors of this article, two of whom are members of a *Medical Research Council* Unit, are engaged on a study of the prevention and treatment of sepsis in industrial injuries at the Birmingham Accident Hospital. It is their thesis that many injuries, instead of being protected from infection from the time they come under medical care, become infected during treatment and that such infection can be greatly reduced by the adoption of a simple aseptic routine in treatment. The source of infection may be the nose, throat or skin of the patient or his attendant, or organisms may be carried from other infected wounds on the hands or instruments of the dresser. In addition to obviously septic wounds, many which appear "clean" may harbour *Strept. pyogenes* or *Staph. aureus*, and so be a potential source of infection. This article sets out some ways in which the infection of

wounds can be minimized in a busy dressing station in hospital or factory.

The chief vehicles of infection are (*a*) hands and pocket-scissors of the dressers, (*b*) instruments, (*c*) dressings packed in bulk and contaminated by hands or instruments when some are removed. To ensure that hands are washed and instruments sterilized after every dressing, the wash-basin and sterilizer should be within easy reach. For a dresser working alone, dressings can be packed separately or collected in a bowl before each dressing is started; but if assistance is available, one nurse should act as "server" and hand out clean instruments and dressings. The bin for dirty dressings should be close to the patient and capacious enough to be used for several hours without emptying.

The ideal routine is: (i) setting out instruments, dressings, etc. on the trolley; (ii) removal of soiled dressings; (iii) inspection and treatment of the wound; (iv) re-dressing the wound; (v) cleaning and sterilizing instruments and bowls; (vi) washing hands; (vii) recording the treatment.

Adherence to this routine does not involve more complicated procedures; on the contrary, work is often simplified thereby. Failure to adhere to it is often due to inconvenience in the arrangement of the dressing station. Plans of well- and badly-arranged rooms are shown in the article, to illustrate the great saving of labour which may follow a careful arrangement. The most important points in arrangement are:

1. The dresser should have to walk only a few steps between patient, trolley, sterilizer, wash-basin and records desk.
2. The paths taken by the dresser and patient should not cross.
3. Good lighting, either natural or artificial, should be provided. For the latter, fluorescent tubular lamps are the best.
4. Where a doctor's desk and chair are provided, a wash-basin should be close to the chair.

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CONTROL OF *STAPHYLOCOCCUS AUREUS* INFECTIONS IN A MATERNITY DEPARTMENT

by F. A. Knott & J. B. Blaikley, *Journal of Obstetrics and Gynaecology of the British Empire*, **51**, 386–400, October 1944

This interesting paper by the Director of the Bacteriological Department, and an Assistant Obstetric Surgeon, *Guy's* Hospital, describes the course of several incompletely controlled epidemics of staphylococcal infection in a maternity department, and the measures which were later effectively imposed to prevent such outbreaks. Between March 1942 and February 1943 five epidemics occurred, two of which necessitated closing the ward. At the end of this time a new régime was introduced, the object of which was, without adding excessively to the labour of the staff, (*a*) to exclude carriers of pathogenic staphylococci, and (*b*) to prevent carriage of organisms from one individual to another. In the following year, with the new routine established, no epidemics occurred and, as a result, it was possible to admit 30% more patients. The maternity department is a self-contained unit consisting of a ward of 24 beds, with separate nursery and isolation wards and its own theatre, kitchen, dairy, etc.

Bacteriological methods: For taking samples from unopened abscesses or bullae aseptic puncture was performed: discharging lesions were first cleaned of superficial material. Nose, pharynx, eyes and vagina were cultured by swab in the usual way. The skin was examined by rubbing with a moist swab or square of gauze which was put into broth and incubated for 5 hours, after which blood-agar plates were inoculated. Dust samples from, e.g. floor and utensils, were taken in a similar way.

Two or three strains of staphylococci were isolated from each primary culture and examined separately. The strains were classed in 4 "grades" according to their cultural and biochemical reactions. Some were also typed by Cowan's technique, but the speed and simplicity of grading by cultural reactions recommended it for routine purposes, and in practice epidemics could be controlled on this basis. Grade A and B strains were considered to be invasive, and patients or carriers with these strains were excluded from the ward. Of 142 strains from infective lesions, 115 fell into grades A and B, while only 5 out of 50 from "normal" skin did so.

GRADING OF STAPHYLOCOCCI BY CULTURAL REACTIONS

	Grade A	Grade B	Grade C	Grade D
Pigment	+++	+++	++	+
Coagulase	++	++	±	∓
Haemolysin	+++	+	±	∓
Gelatin	+	±	∓	−
Mannite	A(24)	A(48)	A(72)	A(72 or ∞)
Lactose	A(24)	A(24)	A(48)	A(72)
Saccharose	A(24)	A(24)	A(48)	A(72)
Maltose	A(24)	A(24)	A(38)	A(48 or 72)
Glucose	A(24)	A(24)	A(24)	A(24 or 48)

A = production of acid; numerals represent time in hours at which reaction appeared

History of the epidemics: All the epidemics started suddenly and subsided gradually in 2 or 3 weeks. In the mothers there was inflammation of the breast, with or without suppuration, or genital infection with slight fever, and in the babies nasal discharge, conjunctivitis or skin lesions varying in severity from septic spots to pemphigus.

In the first outbreak, a grade-A staphylococcus was found in cultures from the nose, throat or hand of 7 nurses and 7 dressers, and from dusting-powder, olive oil and towels used for the babies. The epidemic probably arose from a towel in the maternity-department kitchen which, in breach of the rules, was used by nurses from an adjacent ward containing septic surgical cases.

Soon after a short ward-closure another epidemic broke out. The need for the strict exclusion of carriers had not at this time been realized, and 5 of the previous carriers who had returned to duty were found still to be carrying a grade-A staphylococcus. Three further epidemics were due respectively to a nurse with recurrent colds and hay fever, a patient with a head cold, and a nurse carrying grade-B staphylococci in the nose and on the hands.

After this it was realized that intermittent or incomplete prophylaxis would not abolish epidemics, and that a stricter régime was required.

Prophylactic régime: This was based on the facts which had been revealed by investigation of the epidemics:

i. The infection was usually introduced by carriers.
ii. As masks were systematically worn, direct infection from the skin, and especially the hands, of carriers was probably an important factor.
iii. Feeding-bottles and other ward equipment became more heavily infected than floor-dust.

The following routine was established:

1. Cultures were made from the nose, throat and hands of all nurses before starting work in the department. If a grade-A or -B staphylococcus was found, the nurse was excluded until it had disappeared.

2. Similar cultures were made every fortnight, and at the beginning of a cold, from nurses working in the department. *Staph. aureus* in the primary culture excluded the nurse until it had been graded; if it was of grade C or D she was re-admitted.

3. Similar cultures were made from all new patients and in addition from the vagina and from any inflammatory lesion. The patient was not allowed into the main ward until the primary cultures had been examined and until *Staph. aureus*, if present, had been graded.

4. Masks were worn during attention to patients and babies, and always in the nursery.

5. Before and after attending to patients and babies the hands and forearms were held under a strong spray of water regulated by a foot or elbow tap and dried on a sterile towel which was immediately discarded.

6. Babies were protected from contact with staphylococci, and from the slight injuries to the skin which may form a site of entry, by handling them as little as possible. They were bathed only at birth, at the separation of the umbilical cord and thereafter at intervals of 5 or 6 days.

7. Baths and bathing equipment were thoroughly washed after use.

8. Olive oil, dusting powder, etc., for the babies' use were sterilized daily.

9. Patients and babies with any *Staph. aureus* grade-A or -B infection were isolated.

10. Babies were removed from the ward during visiting hours and visitors were not encouraged. Fathers, if they

wore a mask, might see the babies but were not allowed to handle them.

Results of the new régime: The report covers a period of 14 months after the institution of the new regulations. The most striking effect is set out in a table which shows that, though there were almost always from 1 to 5 cases isolated from the ward because of infection, and some nurses excluded because they were carriers of grade-A or -B staphylococci, yet no epidemic developed during that period. The incidence of infection expressed as a percentage of confinements fell from 43 to 7·7, with an increased proportion of trivial infections. The grading of staphylococci which had been adopted appeared to be justified, as at all times there was an average of 5 carriers of grade-C and -D staphylococci among the staff and these were allowed to remain on duty.

The authors discuss also some tentative deductions made from their investigations. Briefly they suggest (i) that infection of dust and utensils by virulent staphylococci follows rather than precedes an epidemic; (ii) that staphylococci implanted on an infant's skin gradually spread, and that spread is most rapid on moist areas and in flexures; invasion is often determined by a slight injury or area of lowered resistance; (iii) staphylococci are transferred from an infected infant to the hands and nipple of the mother, from where they spread over the body. Inflammation of the breast may follow and the same grade of staphylococcus be recovered from the milk. *Staph. aureus* is often present in milk from a "normal" breast, but in this case it is almost always of grade C or D. No instance of primary infection from the milk was seen.

[*see also BMB* **31**: a review of a report (*J. Hyg., Camb.* 1942, **42**, 474) to the *Medical Research Council* by J. T. Duncan & J. Walker on *Staphylococcus aureus in the milk of nursing mothers and the alimentary canal of their infants.*]

REFERENCE

Cowan, S. T. (1939) *J. Path. Bact.* **48**, 169

*Aerial Disinfection**

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THE USE OF ANTISEPTIC SPRAYS FOR AIR STERILIZATION: A Summary of a Report to the Medical Research Council

by R. J. V. Pulvertaft, *Journal of Hygiene*, **43**, 352–356, April 1944

This is a summary of a report to the *Medical Research Council.* Colonel Pulvertaft, at present serving in the Royal Army Medical Corps, is a Reader in Pathology of the University of London.

The plan of the experiments now described was to create a fine mist of bacteria for 2 minutes in a room of 850 cubic feet [about 240 m.³] capacity by means of an "Atmozon" nebulizer operated by a mechanically driven compressor, and to count the number of viable bacteria which settled on plates of culture medium during 5-minute periods afterwards. The results were compared with those obtained in similar experiments in which the room had been sprayed for 5 minutes immediately before infection with a mist of antiseptic also produced by a nebulizer. An output of 1 ml. of antiseptic per minute was generally used. The test organism mainly employed was a haemolytic streptococcus of serological group C, sprayed from a diluted culture in serum broth.

Of the antiseptics shown to be effective, the following are mentioned:

i. *Eugenol carbinol* is rapid in action and effective in low concentration in propylene glycol. It is easily procurable, but it deteriorates rapidly in solution and is slightly irritating to the nose and eyes.

ii. *Sodium hypochlorite* is inexpensive, non-toxic, highly effective at low concentrations (5 ml. of a 1 % solution in the air of a room of 1,000 cubic foot [about 280 m.³]) and has a deodorant action. The nebulizer must be resistant to its corrosive effect and must not emit large drops of spray which may damage coloured fabrics.

iii. *Hexyl resorcinol* in propylene glycol is effective but slower than the two antiseptics previously mentioned.

iv. *Catechol* is non-irritant and strongly bactericidal in 2·5 % solution in propylene glycol. It is, however, relatively toxic and is likely to prove more useful for horticultural and industrial purposes than for human habitations.

Antiseptic mists were not effective against organisms dried *in vacuo* or shaken into the air from a blanket on which they had been dried. This may be due to their occurrence in clumps and in association with comparatively large dust-particles.

As a solvent, glycerine has the disadvantage of reducing the output of mist, and it cannot be used with hypochlorites owing to the formation of chloroform. Propylene glycol which also cannot be used with hypochlorites is an effective solvent and has itself an antiseptic action.

Antiseptic mists may have a cumulative action, so that an effective concentration may be maintained by the subsequent atomization of smaller amounts of antiseptic. The addition of various surface-tension-reducing agents did not enhance the action of the mists.

Experiments were made to find whether animals which breathed antiseptics in the form of mist or fine powder developed resistance to infection by inhalation. Hypochlorites, hexyl resorcinol, acriflavine, proflavine and sulphathiazole were tried. This treatment had no effect on the incidence or severity of inhalation infection with *Pasteurella muriseptica.*

In conclusion, the author states that a good antiseptic for atomization should bring about 90 % reduction in bacteria with an air concentration of 1 g. in 20,000 cubic feet [about 5,660 m.³]. In the absence of epidemic respiratory disease even crowded rooms contain few organisms from the respiratory tract, and mists in the concentrations used are unlikely to be of value except in time of epidemic disease. Practical points to be considered are: the avoidance of an over-concentrated solution, which may be irritant, or an over-diluted one, which, in reaching effective concentration, may unduly increase humidity; the advantage of several small nebulizers over a single large one, and of continuous or semi-continuous mechanical operation. The author agrees with Baker, Finn & Twort (1940) that sodium hypochlorite seems the most suitable antiseptic for general use under present conditions.

REFERENCE

Baker, A. H., Finn, S. R. & Twort, C. C. (1940) *J. Hyg., Camb.* **40**, 560

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GERMICIDAL MISTS AND VAPOURS IN AIR DISINFECTION

by A. H. Baker & C. C. Twort, *Journal of Hygiene*, **43**, 382–394, September 1944

This is the latest of a series of papers on air disinfection by the scientific staff of the Portslade Research Laboratories, Sussex, England. As the air of rooms and public places is in practice constantly subject to re-infection, an effective disinfecting agent must possess the qualities of persistence, stability and durability. Persistence implies that some at least of the constituents of a mist remain in the particulate state for a given time. Stability implies that the composition of the particles does not change, though their size may alter. Durability refers to active life as a germicide. The authors' researches were mainly carried out in an experimental chamber, 3·03 m.³ in capacity, in which the antiseptic mists and bacteria could be nebulized and plates of culture medium exposed. In the experiments now described, the properties of a number of higher phenols were investigated, particularly in relation to the possibility of obtaining a lasting action. The bacterial mist chiefly used consisted of a strain of *C. xerosis* suspended in broth or saliva. Striking differences were observed in the sensitivity of the phenols according to the agent in which the test organism was emulsified.

The following heat-volatilized germicides were tested: resorcinol, hydroquinone, pyrogallol, pentachlorphenol, benzyl cresol, benzyl phenol, hexyl resorcinol, eugenol

* [*see also BMB* **557**]

carbinol, salicylic acid and benzoic acid. The durability of hexyl resorcinol far surpassed that of any of the other aerosols, and there was little difference amongst the remainder. It was noted that, to obtain a lasting effect, speed of killing of the bacteria may have to be sacrificed. These solutions were made up in methylated spirit, diluted with water if necessary. Different suspending agents, including glycerol, sorbitol, glucose, ethylene glycol and carbitol, were tried, but none of these prolonged the active life of the germicide.

Observation of the effects due to residual germicide on the walls of the chamber led to a study of the effects of germicides evaporated at room-temperature. The agents were applied as a paint on ordinary surfaces, and the duration of their action was observed under various conditions of ventilation of the chamber. Satisfactory destruction of the test organism was obtained, and this was improved by gentle fanning to hasten evaporation. The rate and durability of action were a function of volatility. The details of the experiments do not lend themselves to summarization, but one example may be given. The floor of the chamber was treated with resorcinol paint corresponding to 1,000 mg. per m.^{3} of air-space, and the chamber was allowed to stand with the ports open for 14 days. When the ports were then closed and the test emulsion (*C. xerosis*) in saliva was sprayed into the chamber, the organisms did not survive more than 15–30 minutes.

A number of inorganic substances was tested. Of these, mercuric chloride, atomized mechanically, was effective, but no action was obtained with boric acid or copper sulphate.

While it appears that the actual killing of the bacteria is accomplished by the vapours, in practice it is necessary to dispose small amounts of lowly-volatile germicides as mists in order to attain maximum efficiency. The authors suggest that resorcinol, which has a medium vapour-pressure, is the material most suitable in the first place for a field trial, and experiments in which mice were continuously exposed for many weeks to mist or vapour of resorcinol did not indicate that harmful effects were likely to arise from exposure to suitable concentrations.

This paper contains much information of theoretical and practical interest.

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PROPYLENE GLYCOL VAPOUR AS AN AIR DISINFECTANT : I

by S. W. Challinor & J. P. Duguid, *Edinburgh Medical Journal*, **51,** 280–289, June 1944

The suitability of propylene glycol (1 : 2 dihydroxypropane) as an air disinfectant was indicated by the work of Robertson, Bigg, Puck, Miller & Baker (1942a, 1942b). Challinor and Duguid, working in the Bacteriological Department of the University of Edinburgh, have made further investigations on the efficiency of this substance, observing particularly the duration of its effect in the presence of continuous re-infection of the air in an almost unventilated room of 1,700 cubic feet [about 480 m.^{3}] capacity. The glycol was vaporized in a dish over a naked flame, and bacterial counts were made on air-samples of known volume by means of the slit-sampler described by Bourdillon, Lidwell & Thomas (1941).

In the first experiments a culture of *Chromobacterium prodigiosum* was constantly atomized in a "Dynalysor" by means of which a constant level of bacterial content was maintained after a few minutes. A single vaporization lasting 1–4 minutes of 0·1 ml. of glycol per million ml. of air caused an immediate reduction of viable bacteria in the air, but this did not last more than 10 minutes. Larger amounts caused unpleasant mist-formation in the room. Continuous vaporization of 0·6 ml./million ml./hour could maintain a constant 95 % reduction in bacterial content without objectionable mist-formation, and repeated vaporizations at 10-minute intervals produced the same effect.

When the room was occupied quietly by 16 men, the bacterial content of the air consisted mostly of dust-borne bacteria, only 2–4 % being α-haemolytic organisms, which are used as an indicator of respiratory pollution. Air disinfection by propylene glycol was attempted. In one of a series of experiments it was found that an 85 % reduction in bacterial content could be maintained on the average by continuous vaporization at the rate of 1 ml./million ml./hour. There was, however, some mist-formation and the air had a sweet taste. A lower rate (0·6 ml./million ml./hour) produced no objectionable effects but the degree of disinfection was much less satisfactory. It is noted that on a large scale, for example in hospitals, very large amounts of glycol would be required. There is some evidence that higher concentrations of glycol may be maintained in the air without unpleasant effects by a different type of vaporizer.

REFERENCES

Bourdillon, R. B., Lidwell, O. M. & Thomas, J. C. (1941) *J. Hyg., Camb.* **41,** 197

Robertson, O. H., Bigg, E., Puck, T. T. & Miller, B. F. (1942a) *J. exp. Med.* **75,** 593

Robertson, O. H., Bigg, E., Puck, T. T., Miller, B. F. & Baker, Z. (1942b) *Aerobiology*, ed. by F. R. Moulton, Washington, p. 271

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PROPYLENE GLYCOL VAPOUR AS AN AIR DISINFECTANT : II.

by J. P. Duguid & S. W. Challinor, *Edinburgh Medical Journal*, **51,** 388–395, September 1944

Extending their previous work (Duguid & Challinor, 1944), the authors studied the effect of propylene glycol on the bacterial content of the air of a small, poorly-ventilated room in which a droplet-spray of respiratory organisms was produced and maintained by periodic volleys of simulated sneezes. The sneezing raised the bacterial content by 20 to 300 times, and 15 % to 50 % of the organisms recovered were of α-hæmolytic type. After half an hour, vaporization of propylene glycol was begun and the percentage reduction in the bacteria of the air was calculated from counts made at intervals by means of the slit-sampler [an apparatus described by Bourdillon, Lidwell & Thomas (1941)]. The glycol was vaporized mainly by a device described by the personnel of Naval Laboratory Research Unit No. 1 (1943). This consists of a large heat-insulated beaker in which the output of glycol vapour is produced and controlled by an electric bulb of suitable wattage immersed in the glycol. The recommended output for the room of 1,700 cubic feet [about 480 m.^{3}] capacity was achieved by a 25-watt bulb in a litre beaker.

Experiments in which the operator was the only occupant of the room showed that droplet-infection is more susceptible to the disinfecting action of propylene glycol than dust-borne infection. A very great reduction in bacterial content was readily maintained.

Experiments of the same type were made when the room was occupied by 16 men, who produced a volley of sneezes at intervals for 2½ hours. Bacterial reductions were on the whole slightly less in the crowded room, perhaps owing to differences in humidity. The lowest rate of vaporization of glycol which maintained an average reduction of about 95 % in mouth-spray-borne bacteria was 0·5 ml. per million ml. of air per hour. Vaporization at this rate, which is recommended for practical trial, caused no objectionable changes in the atmosphere.

The bacterial reduction which may be necessary to decrease the incidence of respiratory disease is not known, but there is some evidence that a 10-fold reduction may have a significant effect. Further, the optimum vaporization-rate for any particular set of circumstances may have to be determined by direct trial.

Referring to other air disinfectants, the authors observe that, at the highest practicable dosage-rates, propylene glycol and hypochlorite are of approximately equal efficiency against mouth-spray bacterial infection.

REFERENCES

Bourdillon, R. B., Lidwell, O. M. & Thomas, J. C. (1941) *J. Hyg., Camb.* **41,** 197

[1] Challinor, S. W. (1943) *J. Hyg., Camb.* **43,** 16

[2] Challinor, S. W. & Duguid, J. P. (1944) *Edinb. med. J.* **51,** 280

Personnel of Naval Laboratory Research Unit No. 1 (1943) *Science*, **97,** 208

[1] [*see BMB* **30**] [2] [*see BMB* **587**]

Studies on Wound Infection

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ANAEROBIC INFECTIONS OF WAR WOUNDS IN THE MIDDLE EAST

by J. D. MacLennan, *Lancet*, **2**, 63–66, 94–99 & 123–126, 17/7/43, 24/7/43 & 31/7/43

The author of this paper, which is based on an investigation carried out under the direction of the Deputy Director of Pathology, Middle East Forces, went to the Middle East with a special knowledge of anaerobic bacteria, and has been able to make a comprehensive study of anærobic wound infections which is the first of its kind in the English medical literature since the war of 1914–18. His three papers contain much detailed information and observations both of theoretical interest and of great practical value. His survey embraces the whole of the wounded in the Middle East Forces from the beginning of the war until the second battle of El Alamein, a period in which there were six distinct campaigns.

He first points out that the term anaerobic infection includes several different conditions. Tetanus is deliberately excluded, since observations made on this disease have already been published (Boyd & MacLennan, 1942). Fusospirochaetal infection and the type of spreading gangrene of the skin due to an anaerobic streptococcus and described by Meleney were rarely encountered. Extensive infection of muscle simulating true gas gangrene and caused by an anaerobic streptococcus, always accompanied by some other ærobic organism, is described more fully; 19 such cases were seen. The remaining conditions are due to clostridia of the gas gangrene group, and are divided into 3 categories. Simple contamination of wounds by such bacteria, with some multiplication but no tissue invasion, was seen in 20–30% of all wounded. In about 5% of such cases anaerobic cellulitis developed; this is an infection of connective tissue which produces gas and may be extensive, but is unaccompanied by serious systemic disturbance. The third condition is clostridial myositis, or true gas gangrene, a rapidly spreading infection of living muscle, having an acute and usually early onset, and causing pain, swelling and severe constitutional disturbance. This occurred in about 1·5% of wounds containing anaerobes and 0·4% of all wounds.

Of 164 known cases of gas gangrene, there were 146 in which the author fully analysed the anaerobic flora, and the frequency with which each of many species was found, either alone or in combination with others, is set out in detail, together with an account of the clinical features characteristic of each. *Cl. welchii* was the most frequent, although it did not preponderate over all others as it did among wounded in France in the last war. It causes the classical condition with early onset and abundant gas production. *Cl. oedematiens* came next in frequency; the incubation period is longer, oedema is produced rather than gas, the foul smell may be absent, and a "sense of weight" in the limb is a marked feature. Other clostridia found alone in smaller numbers of cases were *Cl. septicum*, *Cl. histolyticum* (all 10 cases in which this organism occurred, once alone and 9 times in combination with others, were fatal), *Cl. bifermentans* and *Cl. fallax*. The mortality of mixed infections was higher than in those due to a single clostridium, and the possibility of synergic action between them is discussed.

Treatment is fully described. The prophylactic use of sulphonamides and reliance on them alone in treatment were disappointing, but adequate combined antitoxin and sulphonamide treatment appears to have been successful. A study was also made of the flora of desert sand collected at many different points between Benghazi and Egypt, from which it seems that the source of infection must be sought elsewhere. Pathogenic clostridia could much more frequently be cultivated from soldiers' uniforms, and the author regards this as the probable source of infection, and faecal contamination as the cause of its infectivity.

REFERENCE

[1] Boyd, J. S. K., & MacLennan, J. D. (1942) *Lancet*, **2**, 745

[1] [*see BMB* **81**]

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BACTERIOLOGY OF WAR WOUNDS

by R. J. V. Pulvertaft, *Lancet*, **2**, 1–2, 3/7/43

This short paper embodies the conclusions of an experienced bacteriologist after two years' study of infection in war wounds. The author who, before the war, was on the staff of the *Westminster* Hospital, London, held the position of Deputy Assistant Director of Pathology and Officer Commanding the Central Pathological Laboratory of the Middle East Forces.

He begins by insisting on the need for using a standard method of taking material for investigation in any systematic study of wound infection. Samples taken by different methods or inoculated on to different media show widely different results. Again, samples taken from the depth and the surface of a wound differ quantitatively, though generally not qualitatively. In this investigation swabs taken by the surgeon were moistened in the laboratory and then inoculated on to blood-agar plates, the surface of which had been dried with alcohol (to prevent the spread of *Proteus* and *Ps. pyocyanea*). Anaerobic cultures were also made and these were later subcultured aerobically on to blood-agar plates.

The wounds studied are divided into two groups—head wounds and wounds elsewhere. The distribution of the various species of bacteria is similar in the two groups except that *Staph. aureus* (haemolytic and non-haemolytic) was present in 75·5% of 140 head wounds and only 54% of other wounds. In agreement with other investigators the author found that the bacterial flora of a wound might alter considerably at successive samplings. The change frequently synchronized with a change of treatment, and the introduction of certain types of antiseptic for dressing the wounds was almost invariably followed by the appearance of certain species of bacteria. For these reasons the author inclines to attribute the appearance of fresh species to organisms "latent" in the wound and not, in most cases, to infection from outside.

The author goes on to point out that while in this war deaths attributable to sepsis alone are very few (for instance 5 out of 2,472 battle casualties at a military hospital in the Middle East), in the last year of the war of 1914–1918 they were probably no more (for instance a death rate of 0·5% from all causes including gas gangrene is quoted in the Official Medical History of the War). A more important question is whether, by the introduction of sulphonamides and the closed plaster treatment, the incidence of prolonged sepsis has been reduced. Many patients are still seen who, months after wounding, have suppurating wounds, with fever, intractable anaemia and nephritis. The author favours an attempt to reduce the numbers of these patients by relying less on the closed-plaster method and more on the attempt to eliminate bacteria from the wound by persistent local treatment. [At the time this paper was published the first systematic trial of penicillin in war wounds had only just begun.]

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WOUND INFECTION: A PRELIMINARY NOTE ON A COMBINED CLINICAL AND BACTERIOLOGICAL INVESTIGATION OF 708 WOUNDS

by H. L. de Waal, *Edinburgh Medical Journal*, **50**, 577–588, October 1943

The author, who is a Lecturer in Bacteriology in the University of Edinburgh, carried out this investigation in the departments of Surgery and Bacteriology of that University. His object was to follow the bacteriology of wounds and burns from admission to hospital until healing was complete and to correlate it with treatment and clinical progress. The many possible sources of infection of wounds in hospital—air, blankets, throats of patients and attendants and so on—were also investigated bacteriologically. As he points out, 708 cases is a small number where so many variables are concerned and "it is doubtful whether anything short of several thousand would be of real statistical value." He presents here his principal findings and deductions, pending a more complete analysis of the data.

Methods: The swabs were usually taken by the bacteriologist himself. Wounds were sampled just before operation, at each dressing for the next week, and thereafter every 2 to 5 days. Estimates of the numbers of bacteria present

were made chiefly from films. Wounds under plaster were sampled by absorbent threads which were laid on the wound surface and passed cross the skin between layers of vaseline gauze; the gauze passed through a vulcanite funnel in the plaster 2 cm. from the edge of the wound, thus bringing the thread to the surface. Throat-swabs were taken periodically from the patients under investigation and, when indicated, from all the staff and patients in a ward.

Results: The bacteriology of the wounds *at the time of admission to hospital* supported the view now often put forward (e.g. Gissane, Miles & Williams, 1944) that wounds may become infected during treatment if this is not efficiently carried out. The number of wounds contaminated by bacteria increased with increasing delay before admission. Those which had been "cleaned" before admission carried more bacteria of all types than those which had simply been dressed, and twice as many were infected with streptococci. A sterile covering put on soon after injury was the most favourable first-aid treatment. Haemolytic streptococci were more frequent in wounds of the head, neck and upper limb, and coliform bacilli, *Proteus*, *B. pyocyaneus* and clostridia in wounds of the groin, perineum and thigh.

After admission to hospital 86% of all wounds and 67% of burns became infected, the majority with organisms of low pathogenicity; 146 cases acquired a haemolytic streptococcus and 231 *Staph. pyogenes*. On many occasions, from the time the patient arrives in hospital, the wound is uncovered and exposed to potential infection. Among possible sources are the nose and throat of patients and attendants (masks are often inefficient), the skin near the wound, dust from bed-clothes if the patient moves during the dressing, slipped bandages and baths. Of 38 wounds treated in closed plaster, 34 became infected, mainly with non-pathogenic staphylococci and *Proteus*.

Treatment: The author emphasizes that the value of the different antiseptics used locally is difficult to assess because of the large number of variable factors. He gives no indication of the principles on which the wounds and burns were selected for the various treatments. The local applications used were acriflavine 0·1%, gentian violet 1·0%, sulphanilamide, sulphapyridine, albucid 2·5% to 30%, tannic acid and penicillin. The author concluded that penicillin was the most promising of all, but as it was used in only a few cases the results were excluded. Of the rest, acriflavine appeared to be the most satisfactory, as it acted on all kinds of bacteria and was not inhibited by pus. As it tended to delay healing it was best used for 4–5 days and then replaced by another antiseptic, preferably a sulphonamide.

The figures given show that the three sulphonamides all reduced infection by streptococci, but by no other organism. The treatment of burns with tannic acid reduced infection by bacteria of all types, and the author recommends this method for parts where there is little movement, pressure or liability to wetting. For the flexures, sulphonamides in paste or paraffined gauze were satisfactory. The importance of surgical excision of wounds as a means of reducing infection is emphasized.

REFERENCE
[1] Gissane, W., Miles, A. A. & Williams, R. E. O. (1944) *Brit. J. indust. Med.* **1**, 90
[1] [*see BMB* **583**]

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EARLY DIAGNOSIS OF WOUND INFECTION WITH SPECIAL REFERENCE TO MIXED INFECTIONS

by D. McClean & H. J. Rogers, *Lancet*, **1**, 707–708, 5/6/43

This paper by two workers from the *Lister* Institute, Elstree, is a supplementary note on work previously reported by McClean, Rogers & Williams (1943). The earlier paper described experimental work which suggested that the infection of wounds by organisms of the gas-gangrene group might be detected at an early stage before it could be recognized by clinical or ordinary bacteriological examination. The methods advocated depend upon the rapid detection of enzymes produced by bacteria proliferating in the wound. Detailed descriptions of the technique used in these tests were given.

In the present paper, the authors describe the influence of mixed infections by staphylococci or streptococci together with organisms of the gas-gangrene group on the character of the resulting infection, and recommend certain modifications in the tests previously described in order that the nature of the bacterial source of the enzymes may be determined.

It was found that the addition of hyaluronidase-producing staphylococci or streptococci to an infection by non-hyaluronidase-producing *Cl. welchii* or *oedematiens* profoundly modifies the character of the infection. The extension of the infected area is more rapid and, in the case of *welchii* infections, the character of the exudate is altered; it becomes more fluid. Both the staphylococci and the streptococci are found in the exudate in numbers approximately equal to those of the accompanying anaerobe. This is in striking contrast to the failure of either the staphylococci or streptococci alone to initiate a generalized infection. Staphylococcal or streptococcal hyaluronidase is present in high titre in both the muscle extracts and the exudates.

Further observations showed that if a hyaluronidase-producing aerobe accompanies a hyaluronidase-producing member of the gas-gangrene group, then not only will both organisms be found in the tissues, but hyaluronidase from both bacterial species will be present. This will complicate the diagnosis of the bacterial source of the hyaluronidase found in the wound exudate. For this reason, the authors recommend that polyvalent anti-sera should be used in neutralization tests in place of the monovalent sera previously described. Particulars are given of the composition of these polyvalent diagnostic sera.

REFERENCE
[1] McClean, D., Rogers, H. J. & Williams, B. W. (1943) *Lancet*, **1**, 355
[1] [*see BMB* **80**]

593

HYALURONIDASE PRODUCTION BY HAEMOLYTIC STREPTOCOCCI OF HUMAN ORIGIN

by N. Crowley, *Journal of Pathology and Bacteriology*, **56**, 27–35, January 1944

An enzyme, hyaluronidase, has recently been recognized which has the capacity to hydrolyse the mucin-like polysaccharide hyaluronic acid (Chain & Duthie, 1940). This enzyme is almost certainly identical with the "spreading factor" found in certain tissues and produced by some bacteria, particularly staphylococci, streptococci and the gas-gangrene organisms (Duran-Reynals, 1933; McClean, 1936). As hyaluronidase has the capacity to lyse the capsules of streptococci, it appears unlikely that the same strain of streptococcus would produce both hyaluronidase and a capsule. As previous work on this point was conflicting, the present author, working in the Emergency Public Health Laboratory, Cambridge, examined 376 strains of streptococci of known group and type for both hyaluronidase-production and capsule-formation.

Technical methods: For capsule-formation, cultures which had been grown overnight in 20% rabbit-serum broth were inoculated heavily into the same medium, and after 2 hours' incubation the centrifuged deposit was examined for capsules by the india-ink method of Butt, Bonynge & Joyce (1936). In some strains the appearance of the colonies on freshly poured neo-peptone blood-agar plates (Dawson, Hobby & Olmstead, 1938) was also studied.

Hyaluronidase-production was tested by McClean's method (McClean, Rogers & Williams, 1943; McClean, 1943). This depends on the fact that a mixture of horse serum and potassium hyaluronate can be clotted by acetic acid, but that clotting is prevented by hyaluronidase. In these experiments the substrate was composed of equal parts of an 0·2% aqueous solution of potassium hyaluronate (prepared from human umbilical cord) and of horse serum diluted 1 in 3 with distilled water. The streptococci were grown for 18 hours in veal tryptic digest broth enriched with 0·1% glucose. The culture was centrifuged and the supernatant fluid was tested at a dilution of 1 : 10 and, if positive, retested at tenfold serial dilutions. If only the lowest dilution was positive, a further test at a dilution of 1 : 2 was carried out.

Results: A decisive answer to the question posed was obtained in that, of 122 strains which produced hyaluronidase, none showed a capsule, whereas of 264 strains not producing hyaluronidase 186 were encapsulated. In group A

(Lancefield), 308 strains were examined. Although 14 or more strains were examined in each of types 1, 2, 3, 4, 8, 11, 12, 22, 25 and 27 (the remainder being distributed in smaller numbers among 18 other types), in only 2 types were hyaluronidase-producers found—41 out of 47 in type 4, and 23 out of 24 in type 22. In group C, all strains were non-capsulated and 35 out of 55 produced hyaluronidase. In group G all (13) were non-capsulated and all produced hyaluronidase.

The strains tested had been isolated from a wide variety of streptococcal infections or from normal carriers (in a few types only, stock cultures were tested). The author concluded that " there was little correlation between the power to produce hyaluronidase and the virulence of the strain for human beings ". Where strains of the same type of streptococcus were isolated from several individuals in close contact (e.g. in an epidemic of scarlet fever) the characteristics, as regards hyaluronidase-production and capsule-formation, were the same in all.

REFERENCES

Butt, E. M., Bonynge, C. W. & Joyce, R. L. (1936) *J. infect. Dis.* **58,** 5

Chain, E. & Duthie, E. S. (1940) *Brit. J. exp. Path.* **21,** 324

Dawson, M. H., Hobby, G. L. & Olmstead, M. (1938) *J. infect. Dis.* **62,** 138

Duran-Reynals, F. (1933) *J. exp. Med.* **58,** 161

McClean, D. (1936) *J. Path. Bact.* **42,** 477

McClean, D. (1943) *Biochem. J.* **37,** 169

[1] McClean, D., Rogers, H. J. & Williams, B. W. (1943) *Lancet,* **1,** 355

[1] [*see BMB* **80**]

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HYALURONIDASES IN INFECTED WOUNDS

by J. D. MacLennan, *Lancet*, **2,** 433–434, 30/9/44

Hyaluronidase is produced by various species of pathogenic bacteria, including *Strept. pyogenes*, *Staph. pyogenes*, *Strept. pneumoniae* and some of the clostridia (Duran-Reynals, 1933; McClean, 1936; 1941). It can be detected in infected tissues and in inflammatory exudates. The hyaluronidase produced by each organism can be used to prepare an antiserum which is apparently specific for the species of origin. Hyaluronidase can be detected by the mucin-clot-prevention test of McClean and his collaborators (McClean, 1943; McClean, Rogers & Williams, 1943; McClean & Rogers, 1943), and they have suggested that this test carried out on tissues or exudates, combined with neutralization by specific antisera, might form a rapid method of identifying the organism in clinical infections. In this paper Major J. D. MacLennan of the Royal Army Medical Corps reports the results of carrying out this test on material from 51 battle casualties with infected or contaminated wounds, including 12 cases of gas gangrene. The samples of muscle or exudate were taken at operation and tested as soon as possible, usually within an hour.

The organisms present in the wounds included *Cl. welchii*, *oedematiens* and *septicum*, *Strept. pyogenes*, *Staph. pyogenes* and anaerobic streptococci. Six samples gave a partial or slight positive result, i.e. partial destruction of the clot or prevention of clotting at a dilution of 1/1 or 1/2. Only one was definitely positive, preventing clotting at 1/128 and being neutralized by the appropriate antiserum; this sample consisted of pus from an empyema infected with *Strept. pyogenes*.

The author was at a loss to explain the large proportion of negative results—44 out of 51. Two of the strains of *Cl. welchii* which had given negative results in man were tested in animals and gave strongly positive results 12 hours after injection. He points out that the one decisively positive clinical test came from the only case infected by a single organism.

REFERENCES

Duran-Reynals, F. (1933) *J. exp. Med.* **58,** 161

McClean, D. (1936) *J. Path. Bact.* **42,** 477

McClean, D. (1941) *J. Path. Bact.* **53,** 13

McClean, D. (1943) *Biochem. J.* **37,** 169

[1] McClean, D. & Rogers, H. J. (1943) *Lancet*, **1,** 707

[2] McClean, D., Rogers, H. J. & Williams, B. W. (1943) *Lancet,* **1,** 355

[1] [*see BMB* **592**] [2] [*see BMB* **80**]

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DETECTION OF BACTERIAL ENZYMES IN INFECTED TISSUES

by D. McClean & H. J. Rogers, *Lancet*, **2,** 434–436, 30/9/44

Work reported by McLennan (1944) showed that the mucin-clot-prevention test for hyaluronidase was negative in infections in man caused by organisms known to produce this enzyme. In the present work Drs. McClean and Rogers, of the *Lister* Institute of Preventive Medicine, investigated experimentally the reasons for this unexpected result. Hyaluronidase and lecithinase were estimated in muscle extracts and oedema fluid of experimental animals by the methods already described (McClean, Rogers & Williams, 1943; McClean, 1943).

Twelve guinea-pigs were injected intramuscularly with washed 18-hour cultures of *Cl. welchii* to which $CaCl_2$ had been added. Four were injected with 200 units of monovalent *Cl. welchii* antitoxic serum at 2 hours after infection and 4 at 4 hours after infection. The remaining 4 were not treated and were used as controls. Two of each group were killed 6 hours after infection and the rest at 22 hours. This experiment was performed four times with consistent results. The highest dilution at which muscle extracts and oedema fluid of control animals gave a positive result varied between 1 : 16 and 1 : 3848. In 3 out of the 4 experiments, the tests showed decisively that antitoxic serum greatly inhibited or completely suppressed hyaluronidase production. In the 4th there was slight inhibition. Lecithinase production also was inhibited or suppressed by the antitoxin in all experiments.

Reviewing Major MacLennan's 12 cases of gas gangrene the authors point out that several of the negative results can be accounted for either by the administration of antitoxic serum or by the fact that the infecting organism was *Cl. oedematiens*, of which type B produces little and type A no hyaluronidase. But in at least 1 case where *Cl. welchii* was the organism responsible for the infection and no antitoxin had been given the test was negative. It appears that other factors besides the administration of antitoxin modify the results in man. Further animal experiments showed that neither the size of the initial infecting dose of *Cl. welchii* nor the presence of a proteolytic organism such as *Cl. histolyticum* affected the result in animals.

In another series of experiments a streptococcus which did not produce hyaluronidase, *Cl. histolyticum*, and a preparation of hyaluronidase were injected into guinea-pigs separately and in combination. The streptococcus both with and without hyaluronidase was harmless, but when added alone to *Cl. histolyticum* it hastened death, and this effect was increased if hyaluronidase was injected in addition.

REFERENCES

[1] McClean, D. & Rogers, H. J. (1943) *Lancet*, **1,** 707

[2] McClean, D., Rogers, H. J. & Williams, B. W. (1943) *Lancet,* **1,** 355

[3] McLennan, J. D. (1944) *Lancet*, **2,** 433

[1] [*see BMB* **592**] [2] [*see BMB* **80**] [3] [*see BMB* **594**]

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INFECTION OF CAT-BITE AND DOG-BITE WOUNDS WITH *PASTEURELLA SEPTICA*

by E. N. Allott, R. Cruickshank, R. Cyrlas-Williams, V. Glass, I. H. Meyer, E. Straker & G. Tee, *Journal of Pathology and Bacteriology*, **56,** 411–415, July 1944

The authors report 6 cases of septic infection, caused by the bite of a cat or dog, from which the organism *Pasteurella* was isolated. The 3 cases of dog-bite, the first with this infection to be recorded, all occurred in the west and north-west districts of London within 3 months in the winter of 1942–43.

All the infections were characterized by severe local inflammation with pus formation and in three there was subacute osteomyelitis. The *Pasteurella* was several times found in pure culture; at other times it was accompanied by *Staphylococcus*. The three strains which were tested for animal virulence all killed when injected intraperitoneally.

Out of 4 patients' sera tested, 3 failed to agglutinate the infecting *Pasteurella* and the fourth agglutinated at a dilution of 1 : 80.

Methods and Apparatus*

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THE CLEANING AND STERILIZATION OF MILK BOTTLES

by B. C. Hobbs & G. S. Wilson, *Journal of Hygiene*, **43**, 96–120, April 1943

G. S. Wilson, who is Professor of Bacteriology as applied to Hygiene in the University of London, and his colleague, were requested in 1937 by the British Ministry of Health to investigate the bacterial content of milk bottles after treatment in bottle-washing plants in dairies. A full report on their two years' study is available for consultation in the library of the *London School of Hygiene*; the present paper, although containing a mass of information, is only a condensed version of it.

The authors had first to devise a form of cultural test and, having tried several alternatives, decided on the addition of a measured volume of sterile ¼-strength Ringer's solution to the bottle, thorough rinsing, and culturing of aliquot portions in agar. The standard of bacterial cleanliness suggested is a colony count of not more than 600 per pint bottle, i.e. about 1 colony per cm.^{3} of bottle capacity. Their survey embraced 105 plants of 26 different types. The lowest counts were given by bottles from large plants using detergent solutions, application of those solutions by spray being better than soaking in the solution. Steam-sterilized bottles usually gave higher counts and bottles from small hand-washing plants the highest counts of all. The authors favour the use of NaOH as a detergent, and they include data on the strengths of solution required at different temperatures. Immediately after efficient treatment with NaOH, bottles were found to be almost sterile, but subsequent rinsing with water is necessary, and this leads to re-contamination. That this explains many high bacterial counts was proved by the fact that high bottle counts were obtained from plants employing re-circulated rinse-water which itself gave such high counts that bacteria must actually have multiplied in it. If the rinse-water is kept hot enough this cannot occur, but the bottles should also be cooled before being filled. An alternative is the addition of chlorine to the rinse-water: this requires such careful control that the authors think it inadvisable.

The system proposed by the authors is new, namely: a rinse, both internal and external, in water hot enough to remain more or less bacteria-free, followed by *external* rinsing only with re-circulated water which cools the bottle to about 70° F. [about 21° C.]. A final spray, both external and internal, is then given with cold water direct from the main. The paper contains much valuable data and deals with many other practical points connected with the cleanliness and sterility of milk bottles.

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THE SELECTIVE ACTION OF TETRATHIONATE IN BACTERIOLOGICAL MEDIA : A Report to the *Medical Research Council*

by R. Knox, P. G. H. Gell & M. R. Pollock, *Journal of Hygiene*, **43**, 147–158, September 1943

Tetrathionate broth is commonly used as a selective enrichment-medium for the organisms of the typhoid-paratyphoid-salmonella group before plating on solid media. The method is highly successful for the isolation of *Bact. paratyphosum* B and most Salmonellas, but none of the suggested modifications has been so useful for the isolation of *Bact. typhosum* and the suppression of *Proteus* and Morgan's bacillus. The authors, working with the Emergency Public Health Laboratory Service, have made a careful study of the chemical changes occurring in various tetrathionate media and of the effects produced by the growth of faecal organisms in them.

The original medium of Muller (1923) contained sodium tetrathionate, which was formed by the mixture of iodine and an excess of sodium thiosulphate. The authors prepared media in which these three components were present in varying proportions and quantities. Their results are arranged in graphic form. Any excess of iodine caused inhibition of all the organisms tested. Thiosulphate alone has a selective action, but media prepared with it inhibit the growth of the typhoid bacillus while permitting the growth of Morgan's bacillus. Good results were obtained with "balanced tetrathionate" (i.e. prepared by mixing thiosulphate and iodine in the exact quantities indicated by the chemical equation), but the medium ultimately selected for routine use was one in which thiosulphate was in slight excess, containing 25% less tetrathionate than Muller's formula. Details of the preparation of this medium are to be found in a previous paper (Knox, Gell & Pollock, 1942).

The authors made the important discovery that certain organisms, including *Bact. paratyphosum* B, most Salmonellas, *Bact. typhosum* (to a less marked extent) and *Proteus*, but not *Bact. coli*, *Bact. aerogenes* or the dysentery bacilli, can reduce tetrathionate to thiosulphate. This explains, in part, the selective action, as organisms able to reduce tetrathionate have a source of energy not available to the others, and their growth is therefore favoured. The increasing concentration of thiosulphate in the medium also explains why subculture of tetrathionate broth cultures at different intervals of time produces varying results. The reaction involves the production of high acidity which must be controlled by the use of chalk or phosphate as a buffer. Both these substances favour the growth of *Proteus* and Morgan's bacillus, so that the ideal tetrathionate medium has clearly not yet been devised.

The study of solid tetrathionate media gave interesting results. Where thiosulphate is present in excess, the medium becomes opaque. Colonies of *Bact. paratyphosum* B are surrounded by a clear and striking zone of "lysis" caused by bacterial reduction of the tetrathionate. Practical use of these media could not be made, however, as they are too inhibitory to *Bact. typhosum*. The authors recommend a solid medium containing balanced tetrathionate in a concentration of 0·03 molar. This medium has given excellent results in the isolation of typhoid and paratyphoid bacilli and several strains of Salmonella. It is highly selective, but recognition of colonies requires some experience.

REFERENCES

Knox, R., Gell, P. G. H. & Pollock, M. R. (1942) *J. Path. Bact.* **54**, 469

Muller, L. (1923) *C. R. Soc. Biol. Paris*, **89**, 434

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THE LABORATORY DIAGNOSIS OF DIPHTHERIA : A Note on some Present-day Methods

by H. A. Wright, *Edinburgh Medical Journal*, **50**, 737–745, December 1943

The author, who is a member of the bacteriological staff of the University of Edinburgh, discusses some of the difficulties in the laboratory diagnosis of diphtheria, and describes the modifications of technique which have now become current practice in Britain. Modern knowledge demands the use of selective culture-media containing tellurite, and the recognition of the different types of diphtheria bacillus.

It is still customary to inoculate swabs on Loeffler's serum slopes, for this medium promotes the rapid growth of *C. diphtheriae* and good development of metachromatic granules, and will give a positive result with most clinical cases. Swabs from contacts, carriers, and convalescents, however, frequently give negative results on this medium, and this may also happen in mild clinical cases or in severe cases in which other organisms are abundant. Further, the *gravis* type may be difficult to recognize on Loeffler's medium. Petri-dish culture on one of the media containing tellurite, such as that of Anderson, Happold, McLeod & Thomson (1931) or Hoyle (1941), is therefore essential.

Tellurite media inhibit the growth of many organisms common in the throat and nose, and permit the identification of the three types of diphtheria bacilli, *gravis*, *mitis* and *intermedius*, a differentiation which is of considerable epidemiological and clinical interest. The plates are inspected after 18 hours' incubation, but a negative report should be given only after 48 hours, as some strains may be slow to develop. With a low magnification, the dull slate-grey radially striated *gravis* colonies, the smooth round low-domed *mitis* colonies, and the much smaller dark regular colonies of the *intermedius* type can be recognized. Microscopic morphology of

* [*see also BMB* 572 & 573]

diphtheria bacilli from tellurite media can be learned by experience, and the barred forms of the *intermedius* strains are typical. Further biochemical reactions, such as the fermentation of starch by *gravis* strains, complete the typing of diphtheria bacilli.

The bacteriologist appreciates the desire of the clinician for an early and unequivocal report, and this is usually possible in clinical cases, but it must be understood, by the medical officer of health and the hospital administrator particularly, that an accurate final report may be available only after investigations which inevitably take two days, or even longer, to perform.

REFERENCES

Anderson, J. S., Happold, F. F., McLeod, J. W. & Thomson, J. G. (1931) *J. Path. Bact.* **34**, 667

Hoyle, L. (1941) *Lancet*, **1**, 175

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THE FILTRATION OF *MYCOBACTERIUM TUBERCULOSIS* AND *MYCOBACTERIUM STERCUSIS* THROUGH GRADOCOL MEMBRANES

by M. A. Soltys & A. W. Taylor, *Journal of Pathology and Bacteriology*, **56**, 173–180, April 1944

A number of workers have maintained that the life cycle of *Myco. tuberculosis* might include a stage at which viable organisms could pass through filters which would hold back ordinary tubercle bacilli. They claimed that such filtrates, though free of tubercle bacilli of the usual form, would produce a modified tuberculous infection in experimental animals. As the technical difficulties of this kind of work are very great, considerable scepticism has been expressed as to the validity of their results. The evidence is discussed by Topley & Wilson (1936). Various types of filter have been used, but in only one previous paper was work with a filter of known pore size—0·4 μ—described. There was no evidence that a viable form of the tubercle bacillus could pass through this filter (Lewis, Ruckman & James, 1934–35). In the present paper the problem is re-examined by two workers at the *Moredun* Institute, Gilmerton, Midlothian.

Technical methods. Three types of material containing tubercle bacilli were used: (i) cultures of human and bovine *Myco. tuberculosis* from 1 to 35 days old; (ii) lungs and spleens from rabbits dying of experimental bovine tuberculosis, and lymph nodes and lungs from naturally infected cattle; (iii) sputum rich in tubercle bacilli from patients with pulmonary tuberculosis. Cultures of *Myco. stercusis* were also used. The material was ground or shaken with Tyrode's solution [a physiological solution of salts and glucose] to give an appropriate suspension. Preliminary filtrations were made, first through paper pulp and then through Kieselguhr [a preparation of diatomaceous earth].

A culture of louping-ill virus, centrifuged and filtered through Kieselguhr, was mixed with the suspension of tuberculous material as a control for the patency of the filter; 0·1 cm.³ of the filtrates was injected intracranially into mice and in every experiment produced typical louping-ill.

The filters were gradocol membranes supplied by *St. Mary's* Hospital, London, and were of 4 sizes, having a pore-diameter of 2·02 (or 1·95), 1·5 (or 1·43), 1·00 and 0·7 (or 0·69) μ respectively.

Filtration was performed by applying a negative pressure of about 30 cm. Hg for ½ to 1 minute. Each suspension was passed successively through filters of the 4 sizes, samples of each filtrate being taken for testing.

The filtrates were examined for tubercle bacilli by the injection of 3·5 cm.³ intraperitoneally and subcutaneously respectively into two guinea-pigs. *Myco. stercusis* was demonstrated by culture.

The results can be summarized as follows:

i. The bovine type of *Myco. tuberculosis* did not pass through pores of 0·7 (or 0·69) μ diameter in 5 experiments or pores of 1·00 μ in 3 experiments.

ii. The human type of *Myco. tuberculosis* did not pass through pores of 1·00 μ in any one of the 4 experiments performed.

iii. Suspensions of *Myco. stercusis* at 4 dilutions from 1:50 to 1:10,000 did not pass through pores of 1·03 μ diameter.

In the words of the authors—"No evidence was obtained which might have suggested the existence of a form of these organisms in any way analogous to an ultravisible virus."

REFERENCES

Lewis, E. S., Ruckman, M. & James, F. (1934–35) *Proc. Soc exp. Biol. N.Y.* **32**, 645

Topley, W. W. C. & Wilson, G. S. (1936) *The principles of bacteriology and immunity*, 2nd ed., London, p. 292

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AN APPARATUS FOR THE SAFE INOCULATION OF ANIMALS WITH DANGEROUS PATHOGENS

by M. van den Ende, *Journal of Hygiene*, **43**, 189–195, September 1943

Work in the laboratory with certain bacteria, as for example the Brucella group, and certain viruses such as psittacosis, has long been recognized as involving undue risk of infection for the bacteriologist. This danger is liable to be particularly great when experimental infection of animals by the intranasal route is practised; recent experience has shown that the hazard is a very real one when nasal instillation of typhus rickettsiae is made into anaesthetized mice. The apparatus described by Dr. M. van den Ende from the National Institute of Medical Research at Hampstead, London, is designed to overcome this risk. It consists essentially of a steel-and-glass box, inside which the inoculation of the mice is carried out. The anaesthetized mice are passed into the box through an opening in the left side which can be closed by a sliding door. After inoculation they are placed into labelled glass jars held in the right half of the box. The operator wears a surgical gown and rubber gloves and his hands are introduced into the box through rubber sleeves fitted into the front of the box. An essential feature of the apparatus is that draught ventilation through the box is provided by a gas burner in the chimney attached to the box. This prevents the escape of infected droplets from the box during the manipulation of the mice. Above the burner the chimney is so constructed that all air drawn up the chimney is effectively heated. After the mice have been inoculated, they are left in the box for 45–90 minutes, during which period quartz-jacketed ultra-violet lamps inside the box are turned on. The mice are then transferred to cages and the glass jars are immediately sterilized. The construction of the box is described in detail, with the aid of diagrams, in an appendix by A. J. G. Hubbard.

Experiments were made, using *Chr. prodigiosum* as an indicator organism, to determine the degree of aerial infection during intranasal inoculation of mice, and the effect of the chimney furnace and ultra-violet rays on the numbers of infective droplets distributed into the air of the apparatus by inoculated mice. When inoculated mice were kept in the box while the chimney furnace and ultra-violet lamps were not in operation, infected particles were recovered from the air up to 40–45 minutes after inoculation. Ultra-violet rays reduced the number of particles significantly, but the chimney was more effective. The chimney alone was capable of clearing the air in the box almost completely within 7 minutes after the introduction of a heavy spray of bacteria-laden particles. That the draught through the box was effective in preventing infected particles from passing outside the box was shown by sampling the air outside the open door in the end of the box when numerous infected droplets were present inside.

Since the introduction of the apparatus for typhus work in the author's laboratory, only one case of typhus has occurred in 4 months among 8 individuals working on this disease; before the box was introduced several workers contracted typhus within a month of commencing work involving the intranasal inoculation of rickettsiae into mice.

Various Bacteriological Studies

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THE NATURE AND MODE OF ACTION OF STAPHYLOCOCCUS COAGULASE

by W. Smith & J. H. Hale, *British Journal of Experimental Pathology*, **25**, 101–110, June 1944

It has been known for many years that some strains of *Staphylococcus* produce a substance which will cause the

clotting of plasma. Recently this property has been utilized by the bacteriologist in assessing the pathogenicity of strains of *Staphylococcus*, as the substance, coagulase, is seldom produced by non-pathogenic strains (Chapman, Berens, Peters & Curcio, 1934; Christie & Keogh, 1940; Cadness-Graves, Williams, Harper & Miles, 1943).

In routine bacteriological testing, citrated human plasma diluted 1/10 is usually used, but occasional human plasmas are encountered in which no clot, or an incomplete clot, is formed. Rabbit or horse plasma may replace human plasma, but guinea-pig, mouse and fowl plasmas do not clot under the conditions of the test. These individual and species differences, as well as the fact that human fibrinogen after it has been purified by repeated precipitation is not clotted by coagulase, were anomalies which the present work explains. It was carried out in the Department of Bacteriology of Sheffield University.

In preliminary experiments the authors showed that coagulase would pass without loss through a "gradocol" membrane with a pore diameter of 0·50 μ, though retained completely by one with a pore diameter of 0·11 μ. It was relatively heat-stable, as only partial loss occurred in 30 minutes at 80° C. Having established these data, they were able to make sterile concentrated preparations of coagulase by filtration and distillation at 60–70° C. under reduced pressure; with these preparations the remaining work was carried out.

They first ascertained that in the clotting of normal blood all the factors concerned were interchangeable between different species, thus showing it to be highly improbable that differences in the structure of the fibrinogen accounted for the differences in coagulase action in the various species. In the course of this work they discovered that the addition of a testis extract containing thrombokinase (prepared by grinding with distilled water, centrifuging and filtering) to the coagulase-plasma mixture produced clotting in otherwise incoagulable plasmas. Hence it was clear that a second substance, lacking in these plasmas and necessary for the action of coagulase, was provided by the testis extract. Guinea-pig testis was much less effective in activating coagulase than human or rabbit testis, though it was equally rich in thrombokinase. This strongly suggested that the substance which activated prothrombin was different from that which activated coagulase.

A sample of "incoagulable" human plasma was shown to contain small amounts of activator, being clotted by a dilution of coagulase of 1/40, instead of 1/640 which was effective for typical plasma. With both typical and atypical plasmas, the time required for clotting increased with increasing dilution of the coagulase. At the higher dilutions (e.g. 1/50 and over), even the typical plasma clotted with increasing speed if increasing amounts of testis extract were added, while with both plasmas a really large amount of coagulase (1/10 dilution) caused rapid clotting without regard to added activator. The clotting time of highly diluted typical plasma (e.g. 1/50) was greatly reduced by adding testis extract. Thus it appeared that even typical plasmas do not contain activator substance in optimal amount for all doses of coagulase, that a sufficiently large dose of coagulase will compensate for deficiency of activator, and that the prolonged clotting time of highly diluted plasma is due at least in part to the dilution of activator.

The fibrinogen-prothrombin complex of blood can be extracted free of kinase by dilution and acidification of plasma or by repeated precipitation with ammonium sulphate. Such preparations are clotted by coagulase only if activator, e.g. in testis extract, is added.

The combination of coagulase with activator, which may be considered analogous to the combination of prothrombin with kinase, was shown by serial sampling of a mixture of coagulase and testis-extract to be a slow process compared to the rapid activation of prothrombin. Both activator and the activator-coagulase complex were shown to be heat-labile, in contrast with coagulase, which is heat-stable. These facts taken together explain the apparent anomaly that guinea-pig plasma is slowly clotted by coagulase at 20° C., though not at all at 37° C. The small amounts of activator in the plasma react slowly with the coagulase and eventually produce clotting, but only if destruction of the complex is reduced by keeping the mixture cool. Many experiments designed to elucidate the anomaly all supported this explanation.

Thrombokinase and the coagulase activator, both present in testis extracts, can be separated by dialysis, which removes the kinase, or by heat in the absence of electrolytes, which destroys the activator.

From this careful and complete work the authors conclude that coagulase cannot itself convert fibrinogen to fibrin, but that it requires the participation of an activator which is present in adequate amounts in some plasmas but deficient or lacking in others. Further work in progress is being directed towards concentrating the activator and investigating its nature.

REFERENCES

[1] Cadness-Graves, B., Williams, R., Harper, G. J. & Miles, A. A. (1943) *Lancet*, **1**, 736

Chapman, G. H., Berens, C., Peters, A. & Curcio, L. (1934) *J. Bact.* **28**, 343

Christie, R. & Keogh, E. V. (1940) *J. Path. Bact.* **51**, 189

[1] [*see BMB* **148**]

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RELATIVE STERILITY OF THE HANDS OF CERTAIN METAL WORKERS

by I. Lominski & G. R. Thomson, *British Journal of Industrial Medicine*, **1**, 165–167, July 1944

In this short paper from the Departments of Bacteriology and Surgery, Glasgow University, and the Western Infirmary, Glasgow, the authors demonstrate that certain types of work with metals lead to a great reduction in the number of micro-organisms on the skin of the exposed parts of the hands and arms. The workers concerned were handling metal filings mixed with oil, the filings comprising copper, manganese, iron and other elements. Contact with filings alone or with oil alone did not have any sterilizing effect.

Methods: The hygienic conditions and exact occupation of each worker were investigated with great care; those in contact with such substances as bleaching agents, paints and antiseptic soaps were excluded. Forty workers handling metal filings with oil, in whom full and satisfactory investigations were made, were compared with a control group of 86 from various other occupations, including 12 metal workers not in contact with both filings and oil, 22 carpenters and 17 doctors and nurses. With few exceptions the 40 workers in metal filings with oil had only one bath a week and only one hand-wash a day.

At least 2 hours was allowed to elapse between hand-washing and taking the bacteriological samples. Six swabs which had been soaked in sterile saline were used for each individual; areas about an inch square on the right palm, back of hand and outer side of forearm were each rubbed successively with two swabs, and each swab was inoculated on to two blood-agar plates. Colony counts were made and averaged for the 4 plates inoculated from each area. In about 50% of individuals, sampling was repeated after 2 weeks; these results were closely similar and were averaged with the first counts.

Results: The statistical analysis was made on the swabs from the palm, since these were less often sterile and showed less variation in one individual than those from other sites. The counts were classified into 11 groups according to the number of colonies; groups 0–2 contained 0–25 colonies; 3–5, 26–250 colonies; and 6–10, 251 colonies to 5,000 or more. Of the 40 workers in metal filings with oil, 87·5% fell into groups 0–2, and all were in groups 0–5; 12 (30%) gave sterile swabs. Among the controls there were no sterile swabs and 59·2% fell into groups 4–6 (51–500 colonies). Taking group 2 as the limit of "cleanness" it is shown that:

i. the proportion of "clean" individuals among workers in metal filings with oil = 87·5%; among control population = 13·9%;
ii. the percentage difference between the two populations = 73·6%;
iii. this difference is 11·46 times the standard error, which = 6·42%.

In discussion, the authors suggest that this relative sterility arises from the accumulation of fine metal particles in sensitive organisms. They point out also that carpenters showed a slight relative sterility compared with other control groups, and attribute this to antibacterial action by the moulds and related organisms present in wood.

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THE BACTERIOLOGY OF BRAIN ABSCESS

by A. M. McFarlan, *British Medical Journal*, **2**, 643–644, 20/11/43

Dr. A. M. McFarlan, a member of the Emergency Public Health Laboratory Service, reports here a study of the bacteriology of brain abscess, made on behalf of the *Medical Research Council*. He points out that, as the patients had all been referred to neurosurgical clinics, the study does not include fulminating cases, which might present a different bacteriological picture.

Of the 48 patients studied, 36 were male and 12 female; their ages were distributed between 5 and 53 years, with 24 cases between 20 and 40 years of age. Among probable sources of infection ear-disease predominated, accounting for 18 cases.

Films of the pus were stained by Gram and Ziehl-Neelsen methods. In all cases aerobic and anaerobic cultures were made on horse-blood agar plates and in ox-serum broth. When examination suggested the need for enriched or selective media Fildes's agar, 20% ox-serum agar, or horse-blood agar with crystal violet (1 in 500,000) or sodium azide (1 in 1,000) were used.

One abscess showed no organisms in films and was sterile on culture. Of the remaining 47, 33 showed a single organism and 14 more than one. The commonest organism was *Staph. aureus*, found in 15 abscesses (in pure culture in 13) distributed evenly through the various age-groups. All strains were coagulase-positive. Anaerobic streptococci were found in 10 abscesses (in pure culture in 1) and fusiform bacilli with varying biochemical and cultural characters in 10 (in pure culture in 2). The other organisms found (the number of times in pure culture is given in parentheses) were—*Strept. pneumoniae* 6 (6) of which 4 grew only anaerobically in the primary cultures; *Strept. pyogenes* 4 (4), all of group A (Lancefield); *Strept. viridans* 2 (1); non-haemolytic and unclassified streptococci 5 (2); *Proteus vulgaris* 6 (2); anaerobic diphtheroids 2 (0); *Micrococcus tetragenus* 1 (1); and *H. influenzae*, seen alone in films once, but not grown in culture.

The author notes that though streptococci were common they were of several different types which could not justifiably be classed together; the staphylococcus therefore was the commonest single organism. The finding of anærobic streptococci and pneumococci as well as of anaerobic fusiform bacilli and diphtheroids indicates the importance of making anaerobic cultures when investigating brain abscesses. The author points out that it is consonant with experimental work (Wámoscher & Vásárhelyi, 1933; Falconer, McFarlan & Russell, 1943) to suggest that brain abscesses due to anaerobic organisms originate in foci of lowered oxidation-reduction potential (Fildes, 1927) produced by a vascular accident or by aerobic organisms which later die out.

A careful study was made of the degree of encapsulation of the abscesses, but as this was found to vary greatly between the superficial and deep parts even of the same abscess no conclusions could be drawn as to whether encapsulation and bacterial flora were correlated. Anaerobic and Gram-negative bacilli were several times found in abscesses with a well-developed capsule.

REFERENCES

Falconer, M. A., McFarlan, A. M. & Russell, D. S. (1943) *Brit. J. Surg.* **30**, 245

Fildes, P. (1927) *Brit. J. exp. Path.* **8**, 387

Wámoscher, L. & Vásárhelyi, J. von (1933) *Z. Hyg. InfektKr.* **155**, 535

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BIOCHEMICAL CLASSIFICATION OF COLIFORM BACILLI IN SPUTUM

by R. Salm, *Edinburgh Medical Journal*, **51**, 247–251, May 1944

The author, working in the Bacteriological Department of the Royal Infirmary, Edinburgh, made a systematic classification of the coliform bacilli found in sputum. About 1 sputum in 5 yielded coliform organisms, and 100 coliform strains were isolated from 80 sputa. The primary isolation was made from blood-agar and McConkey plates, after incubation for 48 hours.

Coliform bacilli cannot be grouped serologically, as an antiserum seldom agglutinates any strain except that used to prepare it. Pathogenicity to animals is variable. Classification must therefore depend on *in vitro* biochemical reactions, and in this work the following were used: fermentation of glucose, lactose, dulcite, saccharose, mannite, maltose, adonite and inosite; changes in litmus-milk and gelatine; and the "Imvic" reactions, that is (i) indol test, (ii) methyl-red test, (iii) Voges-Proskauer reaction (O'Meara's modification) and (iv) Koser's citrate-utilization test. All the media were incubated at 37° C. for 48 hours, except gelatine which was kept at room-temperature for 2 months.

The strains were classified into 7 groups:

1. *B. coli.* Thirteen strains fell into this group, which consisted of those which gave the 4 "Imvic" reactions (in the above order) as + + − −. All fermented glucose, lactose, mannite and maltose with production of acid and gas, and 9 were motile.

2. *B. anaerogenes.* Distinguished by the fermentation of two or more sugars with production of acid only. Seven strains fell into this group. Five were motile, and 3 liquefied gelatine.

3. *B. proteus-morgani.* Distinguished by fermenting only glucose. Eleven strains, of which 3 produced acid and gas, and the rest gas only. Six liquefied gelatine. Voges-Proskauer negative in all.

4. *B. pyocyaneus.* Distinguished by production of blue-green pigment. Five strains, all motile, fermented glucose with production of acid only, and liquefied gelatine. "Imvic" reactions − − − + were the same for all.

5. *B. faecalis alkaligenes.* Distinguished by not fermenting any sugar. Eight strains, only two of which were motile.

6. *B. friedländer-aerogenes.* This group consisted either of strains which fermented adonite and/or inosite with production of acid or acid and gas, or of those which gave "Imvic" reactions − − + +. Thirty strains fell into the group, of which 17 were mucoid and 7 were motile. Maltose was fermented by all and mannite by all but one; 11 fermented litmus-milk with acid, and 2 with acid and clot; 7 liquefied gelatine. Two of the motile strains gave the above "Imvic" reactions, which are characteristic of true *B. aerogenes.*

7. *B. coli intermedius.* In this group were placed all strains which could not be classified in 1–6. Twenty-five strains; 14 showed motility, 10 liquefied gelatine, 12 fermented milk with acid and 5 with acid and clot. 5 did not ferment lactose. The "Imvic" reactions varied, though all strains gave methyl-red +. They resembled the *B. coli* group in all respects except that the "Imvic" reaction + + − − was by definition excluded.

In addition, one strain which was indistinguishable from a coliform bacillus on primary culture became partially Gram-positive in subculture and proved to be a leptothrix.

In discussion, the author points out that, although the classification was in some respects arbitrary, the great majority of strains fell definitely into one group or another.

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606/89

A HISTORY OF COMPARATIVE ANATOMY

From Aristotle to the Eighteenth Century

by F. J. Cole. London, Macmillan & Co. Ltd., 1944. 524 pages; 200 illustrations. £1 10s. [£1.5]

History books too often prove to be compilations of what is already known, with, perhaps, a little additional matter to bring the work up to date. It is, therefore, especially pleasing to record the appearance of this book, which shows evidence of much original research on the part of the writer. Professor Cole is a former holder of the chair of zoology in the University of Reading and enjoys a world-wide reputation as an authority on the history of zoology. Comparative anatomists, zoologists, and in fact all who are interested in the history of the biological sciences, will welcome this critical review, which traces the development of comparative anatomy from the time of Aristotle to the beginning of the eighteenth century. Although the material is arranged chiefly under the various comparative anatomists, biographical detail is reduced to a minimum and relegated to an appendix, for, as the author himself reminds us, this aspect is one which has been traversed so often before that it is more profitable to confine the text of such a book as this to material which has hitherto partly or wholly escaped attention. This does not mean that the biographical material is inadequate or that the work of the more eminent comparative anatomists receives insufficient attention. The whole story is told and the records of fundamental achievements are linked together with less well-known but nevertheless important developments. The writer has obviously sought widely for his material and has succeeded both in bringing to light some new facts and in correcting some errors. The book is extremely well written and includes some excellent illustrations, a number of which are not easily available elsewhere. There is a useful appendix of biographical notes on all important comparative anatomists, a well-selected bibliography and a full index.

After an introductory chapter on first statements of the comparative method, the remaining 34 chapters are arranged in the following groups: (i) the contribution of Greece; (ii) zootomy down to the sixteenth century; (iii) the development of craftsmanship; (iv) Harvey; the encyclopædists; (v) the new comparative anatomy; (vi) the Dutch school; (vii) academies and societies; the anatomy lesson; (viii) the anatomical museum.

Professor Cole is to be congratulated on the completion of this scholarly work, which will take a permanent place in the literature on the history of science. We can but hope that he will one day provide us with a second volume, bringing the story up to the present day.

606/90

A GUIDE FOR THE TUBERCULOUS PATIENT

by G. S. Erwin. London, William Heinemann (Medical Books) Ltd., 1944. 115 pages. 3s. 6d. [£0.175]

Tuberculosis is one of those diseases in which the intelligent co-operation of the patient is essential, both during and after treatment. For this reason it is necessary to explain to him the cause, methods of diagnosis and treatment, and how he can live safely after the period of his treatment has been completed. Just such an explanation is provided by this book, written by the medical superintendent of Liverpool Sanatorium. As the author points out, the tuberculous patient undergoing treatment hears and comes to use many of the technical terms in daily use around him; such knowledge makes further reading less difficult. The patient who is anxious to learn more about his disease and to co-operate to the full in his treatment will find this book a most useful guide. The writer is careful to explain the meaning of all technical terms employed in the book, and in ten short chapters he discusses (i) the causes of tuberculosis; (ii) how the disease is discovered; (iii) complications; (iv) treatment, general; (v) treatment, special; (vi) home and institutional treatment; (vii) the post-sanatorium régime; (viii) miscellaneous information; (ix) legal and other aspects; (x) non-pulmonary tuberculosis. Particular care has been taken in explaining the post-sanatorium régime, and the physician can safely prescribe this book for his tuberculous patients.

606/91

MINOR SURGERY

by R. J. McNeill Love. 2nd edition. London, H. K. Lewis & Co., Ltd., 1944. 392 pages; 201 illustrations. 15s. [£0.75]

All those practical procedures which can be regarded as "minor" surgical operations are described by Mr. Love and his collaborators. Attention is first of all given to the examination of the patient, and diagnostic procedures capable of being carried out in the consulting-room are described. Sections on antisepsis and asepsis are followed by a description of wound toilet. The principal bandages are described; there is a section on the treatment of hernia. The chapter on minor surgical operations includes descriptions of blood transfusion, shock and resuscitation, cysts, bursæ, tumours, dental extractions, tonsillectomy, removal of adenoids, abdominal paracentesis, the treatment of hæmorrhoids, infections of the hand and foot, amputations. A quarter of the book is devoted to fractures and dislocations, and this contains some excellent illustrations. The book, which is intended as a guide to hospital residents and general practitioners called upon to deal with minor surgical problems and common surgical emergencies, is well produced and profusely illustrated.

Chapter headings: (i) examination of the patient; (ii) antisepsis and asepsis; (iii) preparation of a patient for operation; the operation, after-treatment; (iv) wounds; (v) the vascular system; (vi) cellulitis and abscess; (vii) bandaging, slings, knots, strapping; (viii) hernia; (ix) minor surgical operations (by R. E. Norrish and A. W. Bone); (x) genito-urinary surgery (by A. W. Badenoch); (xi) the eye and ear; (xii) anæsthetics (by D. Blatchley); (xiii) fractures and dislocations (by F. P. Fitzgerald).

606/92

A MANUAL OF DISEASES OF THE EYE

by C. H. May & the late C. Worth. 9th edition, revised by M. L. Hine. London, Baillière, Tindall & Cox, 1944. 538 pages; 371 illustrations. 16s. [£0.8]

This is a concise, practical manual of the diseases of the eye, arranged on systematic lines and intended for the student and the general practitioner rather than the specialist. Whilst it gives all the essential facts on ophthalmology and describes fully the common diseases of the eye, rare conditions and uncommon affections are dealt with as briefly as possible. Notable in the sections on treatment are the incorporation of work on sulphonamide and penicillin therapy, which are becoming increasingly important in ophthalmic practice. Careful consideration is given to vitamin therapy, and attention is called to some tests for dark adaptation, which have come into greater prominence under wartime conditions. Many other changes have been made in this edition, bringing it up to date and in full accord with modern teaching. A number of the illustrations are in colour.

Chapter headings: (i) examination of the eye; (ii) subjective or functional examination of the eye; (iii) objective examination of the eye conducted in the dark-room; (iv) the slit-lamp and corneal microscope; (v) affections of the eyelids; (vi) diseases of the lacrymal apparatus; (vii) diseases of the orbit; (viii) diseases of the conjunctiva; (ix) diseases of the cornea; (x) diseases of the sclera; (xi) diseases of the iris; (xii) diseases of the ciliary body; (xiii) diseases of the choroid; (xiv) intra-ocular tumours; (xv) glaucoma; (xvi) diseases of the vitreous; (xvii) affections of the lens; (xviii) diseases of the retina; (xix) diseases of the optic nerve; (xx) amblyopia and functional affections of the retina; (xxi) general optical principles; (xxii) optical consideration of the eye; (xxiii) errors of refraction; (xxiv) paralyses of external ocular muscles; (xxv) comitant squint; (xxvi) heterophoria; (xxvii) operations on the external ocular muscles; (xxviii) the ocular manifestations of general diseases; (xxix) ophthalmology in the tropics; (xxx) ocular therapeutics; general rules for operations upon the eye; (xxxi) eye injuries in war time; (xxxii) visual requirements for British and Indian public services.

606/93

AIDS TO CLINICAL PATHOLOGY

Including Post-Mortem Technique

by D. Haler. London, Baillière, Tindall & Cox, 1944. 358 pages. 6s. [£0.3]

This small book is an up-to-date combination of two earlier volumes in this series, *Aids to pathological technique* and *Aids to practical pathology*. It has been compiled by the honorary pathologist to *All Saints'* Hospital, London, and is intended for students and others interested in laboratory work. It describes in detail the more common laboratory diagnostic procedures; space does not permit the inclusion of details of the more refined hæmatological procedures or of the grouping of streptococci. Students embarking on the study of clinical pathology will find this useful as an introduction to the subject and for revision purposes. It is particularly important that medical terms in books used by students should be accurate. Unfortunately a number of minor errors have crept into this book; a cursory glance reveals sulphon*i*lamides, Charcot-Leydin, Filde's, Kleb, Schultze-Charlton, *H. pertussio*, Volhardt, Pettenköpfer, Krunkenberg and Krünkenberg, Lancefeld, *F. bancroftii*, Kerne-ikterus. However, the book fills a need and such errors will no doubt be corrected in future editions. It is divided into 54 short chapters, grouped into the following sections: (i) post-mortem technique; (ii) hæmatology; (iii) cytology; (iv) histology; (v) bacteriology; (vi) serology; (vii) parasitology; (viii) biochemistry.

606/94

THE RECORDING OF SICKNESS ABSENCE IN INDUSTRY

(A Preliminary Report)

by A Sub-Committee of the Industrial Health Research Board. Medical Research Council, Industrial Research Board Report No. 85. London, H.M. Stationery Office, 1944. 17 pages. 4d. [£0.016]

The recording of sickness absence in industry makes it possible for industrial organizations to determine the effective labour strength (as opposed to book strength) of their staffs, and enables a check to be kept on the effects of conditions of work on the health of those employed in the various departments of the factory, shop or office.

The *Industrial Health Research Board* has considered various methods of recording absence due to sickness and has suggested a uniform method of recording sickness rates in order to enable valid statistical comparison to be made. After discussing the value of health and sickness records, the report outlines methods of obtaining information and of recording sickness absence. The compilation of a sickness absence return is suggested, to give a monthly, quarterly or annual summary. Sickness absence should be grouped as far as possible into those classes of disease which may have some relation to the working environment, and a nomenclature of diseases for the classification of certified sickness and accidents is provided in the report.

606/95

THE VENEREAL DISEASES

A Manual for Practitioners and Students

by James Marshall. London. Macmillan & Co. Ltd., 1944. 348 pages; 8 coloured plates and 105 illustrations. £1 1s. [£1.05]

The author of this work, who is venereologist to the Eastern Command and a former medical officer of the London *Lock* Hospital, considers that the time is ripe for a general review of the methods of diagnosis and treatment of the venereal diseases. He believes that the average case of venereal disease may well be treated in private practice, and he has, therefore, prepared this full account of methods most suitable for such a purpose.

The various manifestations of venereal and allied diseases are described. Recent advances in the chemotherapy of venereal disease promise more rapid methods of treatment, and the author includes an outline of the experimental work on massive arsenotherapy for syphilis. It is unfortunate that this book was printed before full details of the results of clinical trials with penicillin had become available. It is possible that penicillin, or a similar antibiotic, will prove to be the chemotherapeutic agent *par excellence* for both gonorrhœa and syphilis. Although in syphilis penicillin may supersede massive arsenotherapy, this latter form of treatment has proved very successful, but requires hospitalization of the patient. Modern methods of fever therapy are also described. The sulphonamides, which have become important in the treatment of gonorrhœa, are fully discussed; owing to present difficulties of supply, the author has devoted more space to sulphapyridine than to sulphathiazole and sulphadiazine, although the last two are to be preferred. In the descriptions of routine treatment alternative schemes are avoided as far as possible.

Chapter headings: Part I, gonorrhœa: (i) introduction, (ii–iii) gonorrhœa in the male, (iv) gonorrhœa in the female, (v) aspects of gonorrhœa common to both sexes, (vi) vulvovaginitis in children, (vii) urethral stricture, (viii) the sulphonamides; Part II, syphilis: (ix) introduction, (x) diagnosis, (xi–xii) early syphilis, (xiii–xv) late syphilis, (xvi) congenital syphilis, (xvii–xviii) treatment, (xix) prognosis; Part III, other venereal and allied diseases: (xx) rarer venereal diseases, (xxi) other diseases encountered in venereology; Part IV, technique: (xxii) practical instructions. There are, in addition, appendixes on (i) sociology of venereal diseases, and (ii) list of special equipment; and a short bibliography. The book is well illustrated and includes 8 coloured plates.

606/96

LOCAL ANÆSTHESIA: BRACHIAL PLEXUS

by R. R. Macintosh & W. W. Mushin. Oxford, Blackwell Scientific Publications Ltd., 1944. 56 pages; 33 illustrations. 10s. 6d. [£0.525]

Until 1940 the main disadvantage of brachial plexus block was the uncertainty of success. In that year Patrick described a technique which for the first time enabled the procedure to be undertaken with the assurance that anæsthesia would result. The monograph now under review is written by two members of the Nuffield Department of Anæsthetics in the University of Oxford, and they have based their work on Patrick's method. All the deep structures of the upper extremity, and the skin distal to the middle of the upper arm, are rendered insensitive by brachial plexus block.

After a brief description of the pioneer work of Halsted, Crile, Hirschel and Kulenkampff in the anæsthetization of this region, the authors discuss its indications and advantages, and describe in detail the anatomy of the supraclavicular area and their own technique for the supraclavicular injection of the brachial plexus. The technique is one in which, as the authors themselves point out, the main instruction is better conveyed by pictures than by the printed word and, while the text lacks nothing in detail, the illustrations, beautifully drawn by Miss M. McLarty, are an outstanding feature of this little book. Many are in colour and all, like the text, are well produced on excellent paper. The book is fortified by a few important references to the literature. Its brevity and lucidity make it an excellent example of the short medical monograph; it is an important contribution to the literature on anæsthesia.

606/97

AIDS TO THEATRE TECHNIQUE

by M. Houghton & M. Harding. London, Baillière, Tindall & Cox, 1944. 262 pages; illustrated. 4s. [£0.2]

This latest addition to the *Nurses' Aids* series of small textbooks has been compiled by the sister tutor and the senior theatre sister of *University College* Hospital, London. A good memory and an extensive experience of theatre technique are essential in the theatre nurse, and this little book provides a satisfactory basis on which the student nurse can build her knowledge of theatre instruments and appliances; it will also serve as an aid to memory for the qualified nurse who, without previous theatre experience, may find herself confronted with the task of preparing for an emergency operation. The illustrations are a notable feature of the book; in the chapters on surgical operations are depicted trolleys completely equipped for various operative procedures. The more common positions of the patient on the operating table are also well illustrated. The book is well printed in small but clear type, on good paper. A great deal of useful information is incorporated in its 21 chapters; a glossary of instruments and a full index are provided.

606/98

THE MEDICAL ANNUAL, 1944

edited by Sir Henry Tidy & A. Rendle Short. Bristol, John Wright & Sons Ltd., 1944. 404 pages; 40 plates. £1 5s. [£1.25]

This publication, now in its 62nd year, is an annual review of medical and surgical progress, a digest of the important literature of the world during the previous year. It appeals particularly to general practitioners and others as a means of keeping abreast of recent progress. The very comprehensive series of articles on many subjects is compiled by 41 eminent medical and surgical authorities in Britain. In their introduction, the editors record a number of topics which have aroused more than usual interest during the year under review. We may mention the particular attention paid to infective hepatitis, epidemic nausea and vomiting, mass miniature radiography, rehabilitation. As is to be expected, a great deal has been written during the year on war surgery. An article on ageing (geriatrics) is of interest as indicating the increasing attention which must be given to the problems of old age. Although much had been written on penicillin therapy before this book was prepared, no separate article is devoted to the drug.

606/99

PYE'S SURGICAL HANDICRAFT

A Manual of Surgical Manipulations, Minor Surgery, and Other Matters Connected with the Work of Surgical Dressers, House Surgeons, and Practitioners

edited by Hamilton Bailey. 14th edition. Bristol, John Wright & Sons Ltd., 1944. 628 pages; 724 illustrations. £1 5s. [£1.25]

Pye has been in existence since Lister's day and with the appearance of this fourteenth edition, celebrates its sixtieth birthday. We can recall only one other British medical book (Gray's *Anatomy*) which has survived as long. This edition is a collective work of 80 chapters, contributed by 46 authorities under the editorship of Hamilton Bailey. The editor himself is responsible for numerous chapters, but his most notable contribution is perhaps the profusion of excellent illustrations, an important feature in other works from his pen. A number of these illustrations are in colour; all have been carefully selected with a view to illustrating the technical procedures involved in minor surgery.

Each subject has been carefully revised by its contributor. The advantage of individual contributors for special subjects is obvious, and nowhere better illustrated than in this book, which has the added advantage of expert editorship. Production is of the highest standard, and the index is, as usual, excellent.

This book is an essential tool for the house surgeon and a valuable post-graduate education for the general practitioner.

606/100

A TEXT-BOOK OF GYNÆCOLOGY

for Students and Practitioners

by James Young. Sixth edition. London, A. & C. Black. 1944. 443 pages; 255 illustrations. £1 5s. [£1.25]

This text-book is by the director of the obstetrical and gynæcological unit at the *British Postgraduate Medical School*, who is also professor of obstetrics and gynæcology in the University of London. Besides giving a comprehensive orthodox account of the subject, the book has in the past been noted for its good description of the physiology of menstruation and the sex hormones. A complete revision, both of text and illustrations, has brought this new edition completely up to date and in line with modern teaching. Fifty-seven excellent new illustrations have been added, and some old ones discarded. This is an excellent short text-book of the subject.

Chapter headings: (i) external genital organs, vagina; (ii) uterus, uterine ligaments, pelvic floor; (iii) fallopian tubes, ovaries, parovarium; (iv–vi) menstruation; (vii–viii) history, physical examination; (ix–xi) disorders of menstruation; (xii) the menopause and its disorders; (xiii) disorders of function of procreation, dyspareunia, sterility; (xiv) leucorrhœa; (xv) pain in gynæcology; (xvi) symptoms relating to the urinary system in gynæcology; (xvii) symptoms relating to the gastro-intestinal tract in gynæcology; (xviii) anterior displacements of the uterus, acute ante-flexion; (xix) backward displacements of uterus; (xx) genital prolapse, prolapse of the uterus, cystocele, rectocele, complete tear of the perineum; (xxi) inversion of the uterus, lateral and upward displacements; (xxii) bacteriology of the genital canal in health, physiology of the vagina at different ages; (xxiii) bacteriology of the genital canal in disease, puerperal infection, gonorrhœa; (xxiv) infection of the genital canal through the blood-stream, tuberculosis; (xxv) inflammation of the vulva and vagina; (xxvi) inflammation of the uterus: cervicitis, endometritis, metritis; (xxvii) inflammation of the fallopian tube and ovary, salpingitis and salpingo-oöphoritis; (xxviii) inflammation of the pelvic cellular tissue, parametritis; (xxix) pregnancy in ovary, abdominal cavity, and fallopian tube; (xxx) new growths of vulva, urethra, and vagina; (xxxi–xxxii) fibroids of uterus; (xxxiii) polypi of uterus, elongation of the cervix; (xxxiv) sarcoma of uterus; (xxxv) carcinoma of uterus; (xxxvi) chorionepithelioma; (xxxvii) endometrioma and adenomyoma; (xxxviii) distension cysts of the ovary; (xxxix–xli) tumours of the ovary; (xlii) tumours of the tube and broad ligament; (xliii) development, defects of development, atresia; (xliv–xlvi) vaginal and vulvar operations; (xlvii) abdominal operations; (xlviii) minor gynæcological procedures, douching and tamponing, diathermy.

606/101

ROYAL COLLEGE OF SURGEONS OF ENGLAND

General Annual Report of the Council, 1944

published by the College, Lincoln's Inn Fields, London, W.C. 1, 1944. 28 pages.

This report records the work of all departments of the College during the year ended 31 July, 1944.

On 8 March, 1944, H.M. The King and H.R.H. The Duke of Gloucester visited the College. The Duke of Gloucester was admitted to the Honorary Fellowship of the College by the President, Sir Alfred Webb-Johnson.

Examinations. During the year 26 Fellows and 628 Members were admitted after passing the College examinations. The Council propose to seek power to grant the Fellowship to ophthalmological candidates who pass the primary examination and a special final examination equal in standard and similar in form to the ordinary examination but with subjects mainly ophthalmological.

Sir William H. Collins Chair of Pathology. Sir William Henry Collins has given £100,000 to the College for the endowment of the Department of Pathology and the institution of a chair of human and comparative pathology. Sir William has also arranged to bequeath a further £100,000 for the endowment of the Department of Anatomy and the institution of a chair of human and comparative anatomy. This is one of the greatest gifts ever bestowed on the College. In recognition of this and many other "liberal acts and distinguished labours eminently conducive to the improvement of natural knowledge and of the healing art," Sir William H. Collins was awarded the honorary medal of the College.

Library. Much of the library is still evacuated to the country but a small working library has been open to readers throughout the year. Accessions include 155 modern books and a number of historical items. Some additions have also been made to the list of current periodicals regularly received.

Lincoln's Inn Fields site. The College has acquired additional space in Lincoln's Inn Fields, and hopes that the Royal College of Physicians and the Royal College of Obstetricians and Gynæcologists will agree to come together in combined or adjacent buildings on this site.

White Paper on "A National Health Service". At a meeting of the Fellows of the College, held on 8 May, 1944, the White Paper was discussed. A brief account of the meeting is contained in the report.

The report records awards made, lectures and demonstrations given, and elections held during the year. The research work outlined in the report is given in greater detail in the College's Scientific Report, reviewed in *BMB* 606/102.

606/102

ROYAL COLLEGE OF SURGEONS OF ENGLAND

Scientific Report for the Year 1943-44

published by the College, Lincoln's Inn Fields, London, W.C. 1, 1944. 24 pages.

This report is divided into two sections dealing with (i) museum activities, and (ii) research.

Museum. During the year the museum staff have been principally occupied with the supervision of museum specimens at the various centres to which the collection was dispersed earlier in the war. No museum specimen has been damaged or destroyed from any cause during the period under review, and the Council of the College pay tribute to the authorities of the various institutions to which material has been dispersed for their continued kindness in accommodating such material.

Donations included sets of old surgical instruments and a photograph of the outer coffin which enclosed Hunter's remains, taken after its discovery in the vaults of the church of St. Martin-in-the-Fields in 1859.

Additions to the museum comprised 286 specimens. Twenty-four demonstrations and lecture demonstrations were held in the museum and 14 papers on subjects connected with the museum were published.

Research has been confined to the *Bernhard Baron* Research Laboratories; no surgical research has been possible at the *Buckston Browne* Research Farm since 1940.

Progress is reported in the production of papain-trypsin digests of casein suitable for intravenous administration. In addition, the facilities of the laboratories have been placed at the disposal of the *Medical Research Council* for testing the products of various firms.

The series of experiments designed to test the effect of various sulphur-containing amino-acids on the incidence and severity of liver damage during arsenotherapy for syphilis has been extended, and results obtained have shown that with certain protective treatments the incidence of liver damage can be considerably reduced.

Four cases of acute carbon-tetrachloride poisoning were studied. In such cases acute liver necrosis is apparently the cause of death. One case recovered with methionine treatment; in another, where there was a combination of hepatic and renal dysfunction, methionine and chlorine were both employed, on the assumption that the renal dysfunction was related to choline deficiency.

The value of protein synthesis in hepatic disease has also been demonstrated. There are good reasons for believing that infective hepatitis adversely affects the synthesis of specific antibodies, and methionine and intravenous casein digests rich in methionine appeared to produce good results in infective hepatitis, homologous serum jaundice and severe burns.

Work on nerve regeneration after section and suture has produced valuable information on such diverse problems as the rate of degeneration and regeneration of axons of different sizes, and the relation between the state of the myelin sheath and the onset of function.

606/103

AIDS TO ORTHOPÆDIC SURGERY AND FRACTURES

by I. E. Zieve. Second edition. London, Baillière, Tindall and Cox, 1944. 270 pages. 6s. [£0.3]

This useful volume of the *Aids* series first appeared in 1929, written by E. Crook, of *Charing Cross* Hospital, London, and the new edition has been prepared by the surgical registrar of the same hospital. The book is a short survey of the subject, especially suitable for revision purposes. The greater part of the book is devoted to the locomotor system and deals with the effects of abnormal development, injury, inflammation and new growths; a chapter discusses the effects of these same influences on the nervous system, and a final chapter considers acquired deformities. There is obviously no space in a small book such as this for the full descriptions and numerous illustrations one expects in a text-book, but, in the limited space at his disposal, the author has succeeded in providing a great deal of information concisely written and well arranged. There is a good index.

Chapter headings: (i) congenital deformities; (ii) fractures; (iii) injuries to joints, muscles and tendons; (iv) inflammatory diseases of bones; (v) inflammatory diseases of joints, muscles, tendons and bursæ; (vi) new growths of bone; (vii) paralyses; (viii) acquired deformities.

606/104

TROPICAL MEDICINE

by Sir Leonard Rogers & Sir John W. D. Megaw. Fifth edition. London, J. & A. Churchill, 1944. 518 pages; 89 illustrations. £1 1s. [£1.05]

In the preface to the first edition of this book (1930) the authors wrote: "Considering the number of excellent books on Tropical Medicine which are on the market, the issue of this volume calls for an explanation." No explanation for the fifth edition is necessary, for in the intervening years the book has itself become one of those "excellent books". Both its authors have devoted their lives to the study of tropical disease, and their vast experience is reflected in the book, particularly in those sections dealing with diseases upon which they are recognized authorities.

The book is devoted chiefly to the recognition and management of the commoner diseases of warm climates; it contains only such details of microscopical technique as the general practitioner can carry out in his hospital or study. Special attention is paid to the geographical distribution of the more important diseases. Both authors have spent many years in India, and the diseases prevalent in that continent are given special consideration.

In the fifth edition, much has been rewritten, especially in the chapters on malaria, kala-azar, trypanosomiasis, the typhus fevers, leprosy, and the dietetic diseases. At the present time many additional military medical officers are serving in tropical and subtropical areas; many unfamiliar problems will face them, and this book should prove especially helpful to them. Interesting historical notes interspersed throughout the text prove the wide reading of the authors and tempt the historically-minded reader to refer to

the original accounts of important contributions to tropical medicine. Perhaps in a future edition, the writers will find space for a bibliography of the authorities cited. The book includes a number of good illustrations, and a full index is provided.

Chapter headings: (i) malaria, including blackwater fever; (ii) kala-azar; (iii) trypanosomiasis, sleeping sickness and Chagas' disease; (iv) relapsing fevers, rat-bite fever; (v) the leptospirosis group of diseases; (vi) yellow fever; (vii) the dengue group; (viii) the typhus fevers; (ix) plague; (x) undulant fever; (xi) amœbic dysentery; (xii) amœbic hepatitis and liver abscess; (xiii) bacillary dysentery; (xiv) cholera; (xv) hill diarrhœa and sprue; (xvi) leprosy; (xvii) yaws; (xviii) oriental sore; inguinal ulcerative granuloma; tropical ulcer; Madura foot; dermatomycosis; ainhum; chigger disease; lymphogranuloma or climatic bubo; (ix) ankylostomiasis; (xx) filariasis; (xxi) schistosomiasis; (xxii) snake poisons; (xxiii) dietetic errors in the tropics; (xxiv) dietetic diseases in the tropics; (xxv) climate as a disease factor; diseases caused by heat; diseases caused by light; (xxvi) the incidence of general diseases in the tropics; (xxvii) hints on the use of the microscope; (xxviii) general remarks; diagnosis of fevers; case-taking.

606/105

AFTER-TREATMENT

A Guide to General Practitioners, House-Officers, Ward Sisters and Dressers in the Care of Patients after Operation

by H. J. B. Atkins. Second edition. Oxford, Blackwell Scientific Publications, Ltd., 1944. 311 pages; 60 illustrations. 18s. [£0.9]

When the patient leaves the operating theatre his future welfare depends to a great extent on the kind of after-treatment he receives. This book is written for those who are called upon to undertake the post-operative care of patients. The author, who is assistant surgeon to *Guy's* Hospital, has interpreted "after-treatment" in its widest sense, to include the treatment necessary after such injuries as burns, concussion, contusion; post-operative diet, fluid administration; the control of vomiting; sleep; regulation of the bowels; the care of wounds. Details of the after-treatment of specific operations are dealt with on a regional basis. Particular attention is given to the problem of peritonitis, to the colostomy patient, and to the after-treatment of fractures. Highly specialized operations such as craniotomy for cerebral tumour, lobectomy, thoracoplasty and plastic operations are not included, as the author considers that after-treatment in these relatively new fields has not yet become standardized.

The first edition of the book appeared in 1942 and was well received; in this second edition new chapters on the post-operative treatment of children and on rehabilitation have been added, together with new sections on post-operative coronary thrombosis and the management of the diabetic patient in the post-operative period, and new material in the chapters in genito-urinary surgery and head injuries. The remainder of the text has received careful revision and additions have been made to the illustrations. This is an excellent book, of value to every member of the surgical team. There is a full index.

Chapter headings: (i) early post-operative treatment; (ii) operation wounds, scars and burns; (iii) the ear, nose and throat; (iv) the thyroid and breast; (v) the chest; (vi–viii) the abdomen: (ix) the genito-urinary system; (x) amputations and other operations on the limbs; (xi) fractures; (xii) the nervous system; (xiii) operations on children; (xiv) rehabilitation.

606/106

LONDON COUNTY COUNCIL

Interim Report of the County Medical Officer of Health and School Medical Officer for the year 1943. London, P. S. King & Staples, Ltd., 1944. 30 pages. 6d. [£0.025]

This report is presented by Sir Allen Daley, County Medical Officer of Health and School Medical Officer. Although a slender booklet compared with the pre-war reports, it contains sufficient information to prove the value of the work being carried out so devotedly by the medical staff of the *London County Council* during a most critical period in history.

During 1943 11 of the *Council's* hospitals were damaged as a result of enemy activity. From the beginning of the war to the end of 1943 there were 476 air-raid incidents at these hospitals and 141 patients, 86 hospital staff and 12 ambulance drivers lost their lives. At no time during this period was the reception of the sick and injured delayed and 700,000 patients were admitted to the *Council's* hospitals.

There was a substantial return of population to London during the year; there were more births (45,030) than in 1942 (40,654) and more deaths (39,322 as against 36,057).

The infant mortality-rate was 51 per 1,000 live births (57 in 1938 and 108 in 1918, the fourth year of the 1914–18 war). Maternal mortality (2·13 per 1,000 live births) showed a continued reduction on the figures for the two previous years (from 3·0 to 2·5).

Tuberculosis notifications numbered 5,848 (5,530 in 1942) but the number of deaths (2,460) was about the same. Steps are being taken by means of mass miniature radiography to diagnose the disease earlier. A radiography unit was acquired by the *Council* in 1943 and during October–December 13,334 miniature films were taken, and large films were considered advisable in 433 cases (3·2%); 25% of these showed pulmonary lesions.

The results of immunization against diphtheria are striking During the year, 18,045 children were immunized and it is estimated that by July 1944 68% of London elementary-school children had received protection. Diphtheria cases reported in the schools in 1943 were 562 compared with 3,576 in 1938.

There were 50 deaths from measles which at one time approached epidemic proportions. An influenza epidemic reached its peak in December, when 128 deaths were recorded in 1 week. Deaths from influenza for the whole of the year were 726, the highest since 1937 (198 in 1942).

Scarlet fever was more prevalent and virulent (11 deaths) but there were no notifications of smallpox or typhus fever.

There was a further increase in notifications of dysentery (mostly of a mild type) but deaths did not increase. Enteric fever, a disease spread mainly by the same channels as dysentery, reached a record low incidence of 47 cases, though the deaths numbered 10 (5 in 1942).

There were 205,577 admissions during 1943 to the *Council's* public health hospitals. 13,872 births (13,380 live births) took place in the *Council's* general hospitals and in addition 6,847 confinements at home were attended by the *Council's* own midwives.

The estimated number of elementary-school children in London in January 1943, was 231,634, and by the end of the year this number had increased to about 250,000, with a school attendance rate of nearly 77%. The results of routine medical inspections show that the condition of the children has, in several respects, improved compared with 1938. There is less verminous infestation and scabies, and there are fewer children with enlarged tonsils and adenoids than at the beginning of the war. The condition of the children's teeth is not quite so bad. The nutritional condition of the great majority of children in London still continues to be classed as satisfactory and the general opinion among the school doctors, nurses, teachers and care-committee workers was that there were no signs of any war-time physical or mental deterioration among London school children generally. About 77% of the children attending elementary schools received milk in the schools and about 31% had dinners in the schools.

The following comparisons are made in regard to the children examined in the four age groups in the two most recent war-time years, and those examined in the same age groups in 1938, the last complete pre-war year of medical inspection:

	1943	1942	1938
Numbers examined	96,902	88,325	169,995
	Percentages		
Nutrition unsatisfactory	6·00	6·2	6·6
Nits or pediculi—hair	2·15	1·9	2·3
Teeth—obvious decay	31·01	36·0	29·8
Vision—6/9 or worse	22·61	23·8	32·2
Adenoids and enlarged tonsils	7·6	7·3	9·2
Skin diseases	1·8	2·0	1·0
Enlarged glands	1·2	1·2	1·5
External eye disease	1·9	1·9	1·7
Otorrhœa	0·5	0·4	0·6
Defective hearing	0·3	0·3	0·2
Heart disease	0·8	0·8	1·4
Anæmia	0·3	0·2	0·4
Lung disease (not T.B.)	1·3	1·1	1·5
Tuberculosis (pulmonary)	0·07	0·03	0·04
,, (other lesions)	0·10	0·05	0·02
Rickets (boys—entrants only)	1·4	1·1	1·0
,, (girls—entrants only)	0·9	0·8	0·4

With regard to the *Council's* mental health services, there was a continued fall in the number of mental patients, as was the case in the last war. Particulars given of the results of the work of placing in employment and industrial after-care of mentally defective boys and girls leaving the *Council's* special schools at sixteen years of age show that 10% of them will probably need care in an institution and that the other 90% may be expected to take their place, more or less successfully, in the community.

Over one-third of the members of the regular (peace-time) London Ambulance Service are now in the Armed Forces and their places have temporarily been taken by men and women mainly from the auxiliary service.

606/107

THE LISTER INSTITUTE OF PREVENTIVE MEDICINE

Report of the Governing Body, 1944. Published by the Lister Institute, Chelsea Bridge Road, London, S.W. 1. 1944. 15 pages.

The report of the *Lister* Institute for the year 1944 was presented by the Chairman of the Governing Body, Sir Henry Dale, at the annual meeting on June 19.

Some members of the staff are at present serving with the armed forces or dispersed to Oxford or Cambridge. During the year the *Medical Research Council* established a unit at the Institute for research into, and filtration of, blood plasma and serum for transfusion.

Bacteriological, immunological and serological studies Preliminary trials have been undertaken in the treatment of louse-borne typhus with anti-OX19 serum. Dr. A. Felix has been working on this serum from the horse, and a concentrated serum has been prepared at the *Wellcome* Physiological Research Laboratories. It is hoped to arrange for a strictly controlled clinical trial of the serum.

The typing of typhoid, paratyphoid and food-poisoning bacilli with the Vi bacteriophage has been continued and one hitherto unknown Vi-phage type of the typhoid bacillus was identified during the year as being responsible for a small outbreak of typhoid. This type does not seem to be indigenous to the British Isles. On the other hand, a Vi-phage type of *Bact. paratyphosum* B was found which must be considered indigenous to Britain.

Work has been carried out on dysentery prophylaxis, gas gangrene (including the methods of identification of clostridia), the nuclear structures of bacteria, hyaluronidase and hyaluronic acid.

Biochemical studies. Dr. W. T. J. Morgan and Mrs. R. van Heyningen have investigated pseudo-mucinous ovarian cyst fluids for the presence of the specific blood-group substances. The A substance has been isolated from ovarian-cyst fluid, and experiments have recently been started with the object of finding a method of preventing iso-immunization of the mother by the fœtus, with resulting fœtal death. Work has also been continued on the biochemical action of various bacterial toxins, notably *Cl. ædematiens, Cl. welchii* and *Cl. septicum.*

Studies on gramicidin have been commenced by Dr. R. L. M. Synge. These have for their object the relation of the chemical nature with the biological activities of this antibiotic.

Biophysical and physico-chemical studies have included work on the low-temperature drying of biological materials, on the large-scale processing of human serum and plasma for transfusion, on fœtal and maternal hæmoglobin, and on the osmotic pressure of fœtal and " defatted " serum.

Vaccinia virus. Work on the cultivation of vaccinia virus on the chorio-allantoic membrane of the developing chick, suspended shortly after the outbreak of war, has now been resumed by Dr. D. McClean with a view to preparing sufficient quantities of suitable virus for field trials and for clinical use in vaccination.

Endocrinology. Dr. V. Korenchevsky has continued his studies on the effects of different combinations of hormones and vitamins on adult and senile rats.

Nutritional studies. The Ministry of Food has been anxious to ascertain more accurately the human requirement for vitamin A as derived from preformed vitamin A and from the provitamin carotene. This has been done by means of a human experiment carried out on volunteers at the *Sorby* Institute, Sheffield, by Miss E. M. Hume. Since July 1942 the volunteers have received a diet as deficient as possible in vitamin A and carotene. Observation was specially directed to the measurement of capacity for dark-adaptation and of plasma values for vitamin A and carotene. After about 15 months 2 subjects showed deterioration of the visual threshold, with slight skin symptoms and a very low vitamin-A plasma value. The experiment, which is still in progress, has indicated how large are the vitamin-A reserves in the liver of the healthy human subject.

The nutritive value of the nitrogenous substances of the potato has been investigated by Dr. H. Chick and the nitrogen of the potato has been found to have a biological value at least equal to that of the proteins of whole wheat, weight for weight.

A nutritional survey of women employed in Oxford factories and of local housewives of similar age and social position, carried out from the autumn of 1942 to the end of 1943, showed that the nutritional state deteriorated very slightly during that period, but more so among the factory workers than the housewives.

The capacity for synthesis of vitamin C by young apples on storage has been established by Dr. C. West and Dr. S. S. Zilva.

National Collection of Type Cultures. During the year over 4,000 cultures were distributed and some 200 strains were deposited for maintenance.

Many other investigations are recorded in this report.

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